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ASSESSMENT OF WOUND THERAPY SYSTEMS

Final Report
Contract N00014-81-C-0468
Dynatech Report No. 2165

Submitted to:

U.S. Naval Medical Research and Development Command
National Naval Medical Center
Code 45
Bethesda, Maryland 20014

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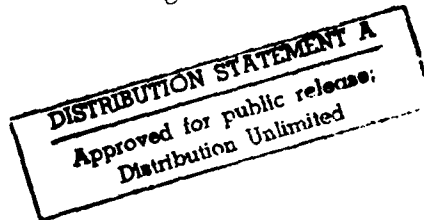
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Submitted:

May 7, 1982



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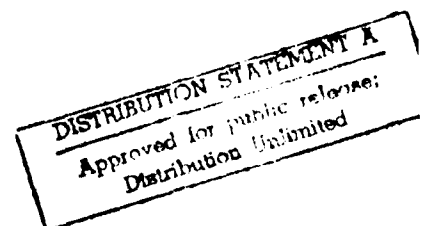


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Section 1

BURN WOUNDS: THE HEALING PROCESS

1.1 Introduction

It has long been recognized that burns cause severe systemic and local disturbances of tissue metabolism and morphology. This section will provide an overview of the complete healing process, with stress on topics relevant to the use of pre- and post-debridement wound coverings. Many of the phenomena of the burn healing process can be affected by the use of such coverings, but there is still considerable room for improvement in such materials and their methods of use.

Areas which will be covered in this section are 1) initial and ongoing circulating changes, and edema; 2) invasive cellular processes; 3) structures of the new tissue matrix; 4) wound coverage via contraction and epidermal migration; 5) immune system changes, 6) toxins released from the burn site; and 7) miscellaneous burn-associated disorders.

This general but broad overview will serve as a basis for a later discussion of present and future desirable properties of burn wound coverings, which serve as a worst-case model of wound-coverings after skin loss.

1.2 Edema, Circulation, and Eschar

1.2.1 Initial Edema

The initial local effect of burn is the establishment of several zones of differential damage and blood flow. Closest to the heat source is a zone of coagulation, with thrombosed blood vessels. Next distant lies a zone of stasis, with blood stagnant but not clotted; finally a zone of increased circulation surrounds this. Very mild burns may lack the second and/or first

zones, whereas severer burns, such as from flame, may possess actual charring as well (39,165).¹

This pattern, established soon after the burn, persists for 24-48 hours in milder burns, longer in severer burns, and results in deprivation of oxygen to tissue in the stasis zone, causing its death. Thus the burn site is actually progressively damaged for at least several days post-burn (165,193). The blood stasis may be partially caused by white blood cells adhering to the walls of venules distant to the burn, thus blocking them (93). The gross manifestation of this stage is edema, which is widespread in >30%-body-surface-area (%BSA) burns. The edema results partly from the local stasis/blockage pressure but mostly from systemic increased vascular permeability. Mediators of permeability are notably released by complement system activation and include histamine, prostaglandins, 5-hydroxytryptamine, and kinins. In the zone of coagulation, treatment with fibrinolytics (intravenously) has led to possible diminution of necrotic area (177).

Although this increased permeability leads to protection by fluid supply to the burn site, it also leads to pulmonary edema and shock (127). Therefore, techniques to control it are of interest. The oldest is cold water cooling post-burn. This is notably effective in preventing histamine release from mast cells at the burn site. Though animal studies indicate cooling may decrease tissue pO₂ levels, it also usually decreases early edema and protein extravasation (46,217). Cooling also leads to increased blood vessel patency retention (260). In animal models, cooling delayed more than 30 minutes post-burn is useless; the sooner the better. However, another study found that cooling delayed only two minutes was followed by impaired resorption of edema

¹The full effects of burns are exhibited at skin temperatures well below those of charring. Considerable work on human models (128,129) has established correlations of thermal exposure skin temperature profiles, pain and magnitude of the expected resultant burns; other animals are extensively used for testing though modeling human skin responses less closely (32,125,139,247,252), e.g., lacking the ability to form blisters.

fluids as late as one week later, and the possibility of creation of a deeper burn by improperly prolonged cooling exists (211). (Deeper burns may cause less edema than shallow ones, hence short term edema measurements do not reveal this.) A definitive study on this point has not, apparently, been done on a porcine model.

The increased permeability of blood vessels at the burn site together with the altered permeability of the damaged epidermis to substances ranging from water to macromolecules (207,265) leads to immediate and continuing fluid losses. It is the intention of immediate post-burn coverings to provide a barrier to stop this huge water loss. (The permeability change might be simultaneously lessened by the (I.V.) use of H₂-histamine antagonists and prostaglandin blockers.) The value of providing such a barrier, whether by immediate or delayed coverings, has long been recognized (32). In addition to the systemic water losses, local dehydration is known to contribute to ischemic burn necrosis (250).

Chemical means have also been tried to regulate blood flow at the burn site. Heparin has been used for some time to prevent burn extension in humans, by countering histamine and proteolytic enzymes at the burn site; it also prevents edema (129, 209, 230).

Methyl prednisolone acetate, topically applied, improved dermal perfusion in a guinea pig study (274). This agent is also used as an antibronchospasmodic post-burn, if necessary (165). The relative importance of various possible steroid effects, e.g., decreasing leucocyte clogging, interfering with prostaglandins and kinins, decreasing chemotaxis, and stabilizing lysozymes, is yet unclear. Cimetidine was recently studied in a mouse model (29); its specific H₂-histamine receptor antagonism was effective in blocking edema if administered pre-burn. However, the clinical significance of these histamine H₂-receptor blockers is still in question (39). Cimetidine is, of course, commonly administered to prevent G.I. ulcers in burn patients. Other specific inflammation blockers have been tried (170,235,246).

In another approach, inhibitors of proteolytic enzymes, the aprotinins contrical and trasylol, were found to reduce leukocyte infiltration and apparently diminish 'secondary necrosis' (45,177). This treatment presumably does not primarily reduce blood-stasis, however.

Histamine is also released from various cell populations by complement activation; preventing its release by de complementation pre-burn reduced murine post-burn shock-lung-type mortality (135).

1.2.2 Long-term Wound Circulation

After the burn site has stabilized, and during subsequent phases of healing (see below), the wound site is supplied with increased blood flow. Of the increase in total body blood flow post-burn, 60 percent is supplied to the wound site, and 20 percent to the liver. This wound circulation is found responsive to α - but not β -adrenergic mediators, and is apparently not immediately demand regulated (by local glucose metabolism)(127). (The increased liver flow is thought possibly epinephrine or pyrogen related.) At the burn site, little or no O_2 is utilized; metabolism proceeds by glucose + acetate, following the preferred pathway for the predominant cell types found at healing wounds (130). It may be that this hugely increased blood flow is required for wound repair: thus, attempting to regulate it, or the non-heat-loss-sensitive portion of the concurrent metabolic rate increase, (18,146) may be a poor idea.

1.2.3 Eschar Formation

Dead tissue at the burn site will spontaneously separate to a removable layer, termed eschar. From animal studies, it is known that germ-free eschar separates extremely slowly (in rats, 14 months vs. 1 month for controls) (144), thus implicating immune activity against invading organisms as a crucial part of this process. Because eschar is a fine growth medium (and infection control difficult in burn patients), its removal, and closure of the burn wound, is highly desirable. Surgical excision of eschar as appropriately early as possible, is becoming widely done; this necessitates a post-excision covering with particular properties (see Section 2). Previously, it was

believed that adherence of such coverings required prior formation of granulation tissue, the highly vascularized layer which supports the normal wound processes of recollagenation and reepithelialization; however, it is now clear that any underlying totally viable tissue may adhere and ingrow into an appropriate covering. In relation to this, an interesting technique is described in which porcine heterograft is used as an indicator of burn depth: it adheres to superficial burns but not to deeper ones which will require antimicrobial therapy and excision (193). Conceivably other (preferably cheaper) coverings, perhaps even an immediate post-burn one, could serve this indicator function.

Research is ongoing in attempts to develop chemical debriders which would eliminate the need for traumatic, bloody, and technically demanding debridement (104,106,107,108). Biologically derived debriders have proved generally slow, disappointing and render the wound far more invadible by bacteria (105,140,144,236), although some animal models appeared effective (89).

Another interesting debriding adjunct is the use of ultrasound to assist cleansing of contaminated surfaces (28).

Lastly, a technique for debridement and cleansing of wound surfaces, particularly effective on infected sites, involves the use of Debrisan® (38,48,51,52,53,190) dextranomer beads. These small (.1-.3mm) beads, sprinkled onto the wound, absorb water and low molecular weight materials, and are claimed to disinfect wounds by 'vacuuming up' free bacteria into their interspaces. Especial advantage is claimed for treatment of hand burns, wherein prevention of crusting aided preservation of mobility. The bead layer is rinsed off and fresh material applied periodically, as necessary. This material would be unsuitable for use with any antibacterial agent. It is not a durable covering, and it certainly does not aim to prevent fluid loss from the burn wound.

1.3 Invasive Cellular Processes

1.3.1 Leukocytes

Immigrating leukocytes play an important role in burn wound healing (39,112). Following the burn, polymorphonuclear leukocytes first adhere to microvascular walls. They then migrate out into tissue, about six hours post-burn.

When they ingest debris, as one destructive mechanism, they produce a large quantity of superoxide and peroxide, some of which escapes into the interstitial fluid. Further interactions due to these materials in the extracellular space are suggested by edema reduction reported with plasma experimentally supplemented with superoxide dismutase and catalase (39).

These neutrophils, of short lifetime, are soon supplanted by macrophages, which differentiate from circulating monocytes upon arrival in tissue. They have a number of functions, including phagocytosis of bacteria, dead tissue (leading to eschar separation) and elastase, digestion of materials intra- and extra-cellularly by lysosomal enzymes, secretion of collagenase, release of digestion products as new substrates for tissue construction, release of autochemoattractants, complement components, interferon, pyrogen, thromboplastin, fibroblast and endothelial mitogens, and prostaglandins (181). Macrophages become activated by lymphokines, immune complexes, and complement product C3b. Their role has been recently reviewed (254).

Macrophage prostaglandin release in particular is implicated in inhibitory growth regulation of lymphocytes and fibroblasts. However, macrophages also produce a factor(s), which, at low concentrations, stimulates fibroblast mitosis. Further work needs to be done to clarify the physiologic and possible clinical usefulness of these factors. Macrophages effect collagenolysis via a complexly regulated enzyme system. Such breakdown may be retarded by various drugs.

1.3.2 Fibroblasts

Fibroblasts are attracted to the burn site by factors produced directly by macrophages, by collagen breakdown products released by the wound, and possibly other materials (40,79,112). Their primary function is the production of new connective tissue, including notably collagen and fibronectin. Fibroblasts from various normal skin layers upon cell culture exhibit widely different average rates of production of fibronectin and procollagen, the most active deriving from the reticular layer (40). They may multiply, and are activated, by various components of the wound milieu (255). Initially, a network of Type III collagen (reticulin), coated with fibronectin, is deposited, which serves as a guide and attachment for more fibroblast movement (113). The mechanics of fibroblast attachment to various types of collagenic substrates (important when considering collagenic artificial coverings) have been extensively studied (116,206), with a resulting better knowledge of fibroblast interactions with artificial in vitro collagens than of the in vivo interactions.

In certain special radiation-burn wound situations, implantation of foreign fibroblasts might serve to speed collagen deposition; however, this is not envisioned as a general clinical treatment (44). Fibroblasts could well serve in vitro functions in artificial covering preparation, however (q.v.)(70).

1.4 Structural Components at the Healing Burn Site

1.4.1 Introduction

The primary structural components appearing at the site of a healing burn (or other wound) include those of normal connective tissue: collagen, elastin, glycoproteins, glycosaminoglycans and fibronectin. In addition, fibrin plays an early role. These will be sequentially considered below.

1.4.2 Collagen

Collagen has been extensively studied, and its synthesis, excretion, fibril formation, binding abilities, degradation, immunogenicity, effects on various cells, and in vitro chemistry, well characterized (197,198, 206). It is special (but not unique)(79) in containing amounts of hydroxyproline which are crucial to its structure. The hydroxylation of the prolines by prolyl hydroxylase occurs after their incorporation into the forming peptide chain (253); this process may be interrupted by lack of vitamin C (110), or by diphenylhydantoin (a Fe^{2+} chelator) (16). In such a case, the underhydroxylated collagen is exuded very slowly and is weak and disordered, slowing the healing process (18,185). Collagen is believed to be secreted into membrane-bound vacuoles, where it forms small molecular aggregates (which increases its temperature stability to denaturation considerably)(189).

Initially, type III collagen (reticulin) is synthesized, which immediately covers fibronectin which has polymerized at the site on fibrin. Although in animal excision studies, collagen Type III synthesis soon ceases (113) studies in human cell cultures suggest a delayed transition to collagen Type I synthesis. Eventually, the fibroblasts produce Type I fibrils, which restores tensile strength to the wound surface. The fibrils are coated with fibronectin (see below), implicated in providing attachment and differentiation control (111,222). As collagen is successively built, it becomes progressively less soluble to salt and acid and finally, through crosslinking (scar maturation) less collagenase degradable (112,189).

1.4.3 Fibrin

Fibrin is essentially the first protein to form a structure at the burn site. Its polymerization (i.e. clotting) is triggered by its contact with the native extravascular collagen (all collagens are variously hemostatic)(221), and bonding to it is responsible for the initial adherence of artificial coverings (161,195). Failure of coverings to adhere to contaminated wounds is associated with the bacterially activated conversion of plasminogen to plasmin, which then degrades any fibrin present. In addition, bacterial proteolytic

enzymes can directly degrade fibrin. The fibrin degradation products then inhibit further thrombin-catalysed conversion of fibrinogen to fibrin. Attempts to boost fibrin synthesis, by thrombin and fibrinogen local supplementation, or to block fibrin degradation, by a protease inhibitor (trasylol), were not successful (86).

Although fibrin-based adherence of artificial coverings is presumably covalent, apparently purely non-polar adherence of cyanoacrylate-type adhesives to various tissues may be achieved by benzoylating the surface, probably the 3-amino lysine groups, before or along with adhesive application(9). This technique is not utilized by any current immediate or post-debridement burn covering.

1.4.4 Fibronectin

Fibronectin, known historically by such names as cold insoluble globulin, LETS (large, external, transformation sensitive) protein, and α_2 surface-binding (SB) protein, is a component of plasma and tissue whose broad functions have only recently been under investigation. It is implicated in cellular growth-control, transformations, morphology, aggregation, and adhesion (203,220). It is present in normal skin as a component of the basement (cell exterior) membrane. It is synthesized by fibroblasts, and is also present in plasma. It is generally believed that all these fibronectins are identical; certainly they are very similar(201).

Fibronectin first plays a role in the healing burn by coating the fibrin at the site. This step, and subsequent linkage of the fibronectin layer to collagen, are effected by serum factor XIIIa (50,111). It has also been suggested that attachment of cells to native collagen is dependent on glucosyl-transferase, not fibronectin (see Section 1.4.5)(206). The subsequent transition of the wound matrix from collagen-III/fibronectin + fibronectin/collagen-I has already been mentioned above. The relative contribution of fibronectin at the wound site from plasma vs. from fibroblast synthesis is as yet unclear (261), but its primary functions there are well known. These are 1) serving as a substrate for epidermal, endothelial, and fibroblast cell migrations (266),

2) cueing the morphology of the developing tissue (206), and 3) promoting phagocytosis by macrophages and fibroblasts (261). In connection with this last, the systemic effects of fibronectin, as liver phagocytosis effector, and increaser of cardiac output and index, O₂ delivery and consumption, and peripheral and lung blood flows, deserve mention. Serum depletion of fibronectin post-burn indicates an as yet clinically unexploited role as a supplement for this protein (103).

The ability to attach to cells is central to fibronectin's function.* The mechanism is as yet unclear, but may be dependent on regulation of configuration on a surface by crowding from other binders, such as albumin, and only already-surface-attached fibronectin affects binding-receptors on (other) cells (162). Presence of fibronectin is hypothesized to trigger the asymmetric distribution of membrane molecules which cells use to induce normal structural differentiation.

In connection with phagocytosis promotion, fibronectin may play an important part in scar remodelling, by serving as a marker of damaged, or in any way unmaturing, collagen (261).

Finally, additional importance of fibronectin in regulating wound repair is suggested by a finding that antibody to fibronectin, applied to fibroblasts, raised their rates of mitosis and glycosaminoglycan synthesis (147).

1.4.5 Glycoproteins and Glycosaminoglycans

Glycoproteins and the glycosaminoglycans (GAGs) they bind are normal components of the extracellular matrix. Hyaluronate is the major glycoprotein, and post-burn is degraded and remodelled, leading to erroneous conclusions when attempting to deduce collagen synthesis by following collagen

* Fibronectin is not highly selective, it also binds tenaciously to tissue culture dishes.

as percent of total wound proteins (a prevalent former practice)(79). The glycosaminoglycans of skin, as measured electrophoretically, are not immediately altered by a third degree burn, suggesting, again, the possibility of minimizing burn damage by appropriate therapy (264). Glycoproteins and GAGs are known to affect various cell processes; most important in untreated burn healing may be that of (along with fibronectin) regulating attachment and detachment of cells, notably highly mobile ones (50,202). GAGs have been practically used to speed dental healing and decrease its pain (169); more recently they have been used to immuno-inactivate collagen in post-debridement burn coverings (3,15,183).

1.4.6 Elastin

Elastin is the protein responsible for skin elasticity. It is of some small note for its sparsity in the late burn scar. Myofibroblasts may function as a replacement for it in the healing wound, trying to place the collagen network under contraction. Elastin also is one of few proteins (besides collagen and complement) to contain significant hydroxyproline(79,267).

1.5 Wound Closure

1.5.1 Introduction

Closure of the (untreated) burn wound takes place by the processes of contraction and epidermal migration. Contraction is the process of bulk skin movement from the edges, drawn in by active work by myofibroblasts; migration is the separation and movement of activated epidermal cells over the wound surface.

1.5.2 Contraction

Contraction is accomplished by a specialized fibroblast, the myofibroblast. This cell, which actively contracts the forming collagen network by a process similar to muscle action, is known to require a properly formed network upon which to act; weak and disordered collagen produced by prolyl hydroxylase inhibition, or by prevention of its crosslinking by lathyrogens,

retards contraction and inhibits development of wound tensile strength; conversely, topical treatment with prednisolone acetate hastens wound strength and might be expected to hasten contraction by forming adequately mature collagen sooner (16,182). A trade-off in which more exuberant, but less organized, collagen was produced, by local injection of E. coli endotoxin, led to net unaffected wound strength (100). Another possible route to collagen disorganization, by providing proline analogues which will be unhydroxylatable, is under investigation (91).

Contraction per se leads to some scarring; thus there is incentive to try to speed healing in ways which increase epidermal migration and decrease contracture (18,35). Post-debridement burn coverings attempt to do this by providing a matrix which lends to less myofibroblast activity. It has been suggested that contributing to this process is the ability of the immature collagen fibrils to adhere strongly when opposed by normal skin buckling. (This process is presumably unrelated to normal skin tensile-strength development, which is promoted in rats by exposure of incised wounds (172).) This may lead to contractural scarring at joints (182). Perhaps Jobst compression garments prevent any such buckling and consequent gluing. If so, collagen maturing agents/cross-linkers might hasten the whole process and permit shorter obligatory Jobst use. One study of thyroid T3 supplementation in rats, indicated that softer, flatter, and more supple scars were obtained in excised, then subsequently exposed or grafted sites (240). Since T3 acts more or less generally, speeding all metabolic processes, more specific agents would be of interest. In human burn victims, T3 and T4 levels are usually normal post-burn, (although production and clearance are elevated) (127), except for lowered T3 in moribund patients. In this event supplemental T3 is thought to aid wound healing and leukocyte mechanisms (127). Animal study of this area is difficult since no animals mimic man's ability to hypertrophize a scar (267).

1.5.3 Epidermal Migration

Epidermal migration, a process of deinhibition of mitosis and movement, is thought to be regulated by decreased chalone and/or catechola-

mines (112) and by specific growth factors present at the wound site (epidermal and fibroblast growth factors-EGF and FGF)(these will be treated in Section 3). Various other materials have been investigated which may stimulate epidermal mitosis (85,115,179,231,262). Salient points for burn wounds include 1) the necessity of keeping the site wet, if even by only mild occlusion (114), as dehydration kills epidermal cells; and 2) production of inhibitors by the burn. Crude extracts of (burnt vs. unburnt) guinea pig skin drastically affected DNA synthesis in epidermal cell culture (54). Isolation of various toxins from burnt skin is an active field (see also Section 1.7.2 on burn toxin)(181).

Epidermal migration has been reportedly aided by fine-mesh synthetic materials, in the (somewhat crude) exposure method of wound healing. These materials are postulated to give epidermal cells a scaffolding for attachment as they move (87,131).

1.6 Immune System Disorders

1.6.1 Introduction

The recovery of burn victims is threatened by bacterial invasion; degenerative changes which occur in the immune system post-burn greatly exacerbate the problem. These changes (treatment of which is more systemically than locally feasible) may be broken down into three areas affected: the complement and immunoglobulin system (humoral immunity), the lymphocyte system (RES), and the leukocyte system.

1.6.2 Humoral Immunity Changes

The immunoglobulins and complement system are variously impaired following burn injury (for an account of normal function see (275)). Plasma levels of all five immunoglobulins are decreased post-burn until days 5-15 (variously). Although some toxins are directly neutralized by IgG, mostly the decrease is due to triggering of the complement cascade.

Alternate complement ability is decreased in all burn victims, while classical pathway activity declines only in septic patients (27,39). The alternate pathway activity may be restored by properdin supplementation, with possibly beneficial clinical effects. Heat stable opsonin and agglutinin titres are normal post-burn, and heat labile opsonins are normal even in septic patients (27,127). It is thought that alternate complement depression post-burn is not clinically serious and may be due, in addition to lowered C3 levels, to an as yet unisolated inhibitor to C3 conversion (39,263).

1.6.3 Lymphocyte Changes

In order to produce antibody, three mononuclear cell types are sequentially involved. First, the facilitory macrophage alters an antigen, which can then activate a helper-T cell. The helper-T then acts on B cells, stimulating them to produce antibody (102). This finely tuned apparatus is controlled by the presence of inhibitory macrophages and suppressor T-cells as well. Complement activation places the facilitory macrophages in operation; foreign proteins alone may actually stimulate suppressor T-cells.

The normal functioning of this system biochemically is incompletely known; it has been suggested that two compounds are central: interleukin 1, from macrophages, whose major function is to induce maturation of T-cells (thus sensitizing them), and interleukin 2, possibly an immature T-cell product, which stimulates proliferation of killer- and helper-T cells after their previous exposure to mitogens/antigens (153). The highly cytotoxic killer-T cells may feedback inhibit immune response by competing with proliferating T-cells for cellular antigen (216). One further substance, "Migration Inhibition Factor", released by T-cells, causes macrophages to stop moving and start phagocytosing (139).

Because this system is feedback controlled, burn-induced changes, it was thought, could occur by increases of inhibitory feedback, as well as decreases in stimulatory impulse. This has been amply confirmed; the 'improper' inhibitions are thought the dominant cause of post-burn

immuno-incompetence. Although control of inhibitory ϕ (ϕ = macrophage) (as by indomethacin) may be possible (132), most efforts have concentrated directly on the increased activity of suppressor T, because mononuclear populations from immunodepressed patients washed free of suppressor ϕ , still remain inactive. Here immunodepression, which indicates lack of defense to, and therefore immanence of, fatal sepsis, is measured by the lack of mitogenesis of T-cells upon phytohemagglutinin (PHA) challenge (139,194). This test predicts a poor prognosis well before measurable sepsis (the onset of which would be masked by the normal burn hyperthermia). Research into mechanism of the suppressor-T-cell activation, and how to reverse it, is ongoing (218). Weak sensitizers to PHA- and conA-challenge, such as arginine (136), are unlikely to be useful; the use of potent agents used in other immune diseases, such as thymic peptides (149), are as yet unreported.

1.6.4 Leukocyte Changes

Leukocytic competence is compromised following burn, as measured by the chemotaxis, random movement, and phagocytotic abilities of polymorpho-nuclear leukocytes (PMNs). Initially, (through the second week post-burn) these changes might be due to complement depletions; later on, other mechanisms, such as inhibition by high catecholamine levels (clinically reversible by levamisole) (127) or by other serum inhibitors (39) must be invoked. These changes are considered of secondary importance to lymphocytic inhibition.

1.7 Other Effects of Burn Wounds

1.7.1 Burn Toxins

Burns exert several other effects on the body, of which a select few are listed below. First, let us consider the matter of a dermal "burn toxin."

A specific toxic lipoprotein material is thermally generated at the burn site, even at temperatures below these irreversibly denaturing skin

protein. The toxin appears, by EM, to be formed by trimerization of a non-toxic endogenous skin monomeric subunit. This precursor has an apoprotein core of \overline{MW} 454,000, interlaced with several lipid materials, totalling \overline{MW} 10⁶ (22,42,63).

The burn toxin lipoprotein has been isolated and its effects on rat liver tissue studied. Severe degenerative ultrastructural mitochondrial changes, producible by the pure toxin, are observed in days 5-7 post-burn, (i.e., well after shock has been controlled in the burn victim), along with lowered ATP:ADP ratios, and impaired abilities to release glucose, urea, and amino acids.

Intravenous ATP restores the ATP levels of the liver and heart, and reverses morphological liver changes (17)(local beneficial effects of ATP in post-burn cultured tissue have been known since 1971)(238). However, this study was done at only three days post-burn, and hence ATP may not have been counteracting the lipoprotein-burn-toxin's effects. It has also been suggested that the toxin could be bound at the burn site, as by topical cerous nitrate, until removed by debridement. This possible, minor function of a temporary burn wound covering has not yet been investigated.

If this toxin is not immobilized or inactivated (239), it might be affecting other targets in the burn victim's body, such as erythrocytes (see next section). Due to other effects of burn, namely collagenolytic serum activity, the blood-brain barrier becomes more permeable. Access of this toxin (or others), to produce neurotoxic effects, could thereby result (109).

1.7.2 RBC Disorders

Abnormal erythrocyte morphology, and lysis, is produced by large burns. In a 50 percent body surface area burn, for example, a 10 - 15% loss of RBCs is seen, necessitating mannitol to preserve kidney clearance (165). Percent hemolysed blood post-burn was studied in rats: although injected hemolysed blood at 2.8 percent circulatory volume just

induced RES depression, a burn severe enough to cause 3.1 percent hemolysis caused such severe RES depression that 80 percent mortality occurred. This suggests that hemolysis alone in rats is not the primary RES depressor (259).

Besides hemolysis, a partial irreversible inability of RBCs postburn to maintain a Na^+ gradient to extracellular fluid has been observed (145). Other membrane changes of the RBCs include spiculation, which has been (unconvincingly) attributed to decreased blood albumin and elevated free fatty acids (269).

Section 2

IMMEDIATE POST-BURN COVERINGS

2.1 Introduction: The Purposes of Immediate Post-burn Coverings

As discussed previously, the burn wound is in an unstable condition for up to several days post-burn, and immediate protection of the site to prevent dehydration and bacterial contamination may be of considerable clinical value, both to the burn site locally and systemically by lessening fluid losses (82). Based on considerable work that the lipid content (specifically linoleic acid derivatives) of the stratum corneum was responsible for the water-barrier properties of epidermis, it was natural to investigate the use of lipid materials post-burn to restore such a barrier. It is to be noted that such replacement was only intended to counteract water loss post-burn, not to provide a bacterial barrier. Animal and human tests of a preparation incorporating ethyl linoleate and various additional stabilizers (called hELate®) were run, finding that a single application in animal burns resulted in faster healing with less contracture than untreated controls. However, side effects of hepatic and renal damage, particularly in conjunction with use of antibacterials, rendered its safety questionable. The investigators blamed impurities in their ethyl linoleate, unknown components of Silvadene, and oxidation products of ethyl linoleate (hydroperoxides and epoxides) as causes, without confirmation; it is possible that the last of these are formed enzymatically in the burn wound and are hence unavoidable with this treatment (174,175,244). On the basis of some human trials, in which hELate was said to provide pain relief, sooner epithelialization, better eventual pigmentation and hair retention, and to allow fewer eventual grafts, its use was recommended. This study however did not have a proper control group, and further reports on this material have not recently appeared (since 1976)(205). It was unknown if ethyl linoleate exerted any beneficial effects other than on water retention.

Given the limitations of this treatment, it was natural to seek materials that provided a simultaneous water and bacteria barrier.

Possibilities examined up to present have included dry and wet films and foams of various materials, applied as wipe-ons, spray-ons and slap-ons. This study focuses on coverings firm and adherent enough to be used without additional dressings. These materials are here divided for convenience into dry (non-gel type) films, biological gels (gelatin and gelatin/pectin materials), synthetic hydro-gels, odd materials such as complex ionic gels, and adhesives.

2.2 Dry Films

Let us consider these possibilities in order of composition; first, dry films. In order to adhere, a dry (i.e., waterless or low-water content) film must be applied in a form that can initially bond to the eschar. A notably successful material at this is poly (ϵ -caprolactone), which may be dissolved in a quick drying solvent and applied to the burn site. An extremely adherent, although very thin, dry film forms. Incorporated plasticizers improve the pliability of this material, and in animal tests, it exhibited adequately low water vapor transmissions (for a .05 mm film, 1.4 mg/hr \cdot cm² comparing with ca. 0.6-1.9 mg/hr \cdot cm² for normal human skin from various body locations) (for testing methods see 37,60,66, 142,148,241,247). One drawback to this treatment is the solvents (acetone, tetrahydrofuran, or, in current testing, methyl acetate/methylene chloride) employed which would cause additional tissue damage or pain upon application to a conscious patient (12,13,157).

Another type of plastic non-gel film used for burn/wound coverage is that based on poly(ethoxyethylmethacrylate) or p(HEMA). Two current European products, Novitas' Wound Spray and Nobecutan (Former US trade name, Rezifilm), incorporate this plastic; the former also contains methyl methacrylate. These two sprays differ in their solvents: the Novitas spray uses a Freons mixture as solvent and propellant; the Nobecutan contains ethyl acetate (capable of causing pain and tissue damage)(10). Both of these sprays incorporate antibacterial agents. One might imagine that such sprays should produce faster healing than in uncovered controls;

however, no acceleration of excised wound closure is seen, and the only induced effect seems to be higher wound tissue enzyme levels 4-8 hours after application (34).

Along the same chemical lines, a spray of poly(butylmethacryl acetate) was tested, which resembled Nobecutan (171).

Nobecutan has been referred to by one critical reviewer as a 'modern collodion' because it converted infected wounds into abscesses. It was also downgraded for painfulness of application (caused by the solvent)(67). Because of the destructiveness of the solvent, U.S. use of this material ceased by the early 1970's. However, sprays propelled and dissolved by Freons should have no such restrictions to their use.

Other drying-type films have not been recently investigated; only back in 1969 was a poly (amino acid) film study done (223), probably deriving from very early "Zinax" Casein/Zinc burn salves (229). Such a film would be rather biodegradable; in any event, it cracked in use.

2.3 Biological Gels

A second type of immediate covering is that based on biological gels. Gelatin-type materials of recent note include a gelatin-incorporating foam, and a solid gelatin/pectin type adhesive wafer. The foam material incorporates animal gelatin (which may be glutaraldehyde cross-linked), a Fe^{2+} (ferrous) salt, a bactericide, water, surface-active agents, and drying aids, such as alcohols. The only advantage claimed reasonably for this foam is the continuous irrigation of the burn surface by drainage from the foam for some hours after application; this is alleged to wash away surface bacteria, although to where is not immediately clear. The function of the Fe^{2+} ion appears to be to oxidize to Fe^{3+} and as such stabilize the gelatin in the foam against bubble collapse. One disadvantage is the thickness of foam required: 3 cm of dried foam are needed to allow a water transfer of 3.3 mg/hr-cm^2 . This would appear reasonable, except that the apparatus and conditions used for testing permitted only $6.6 \text{ mg-H}_2\text{O/hr-cm}^2$ to pass

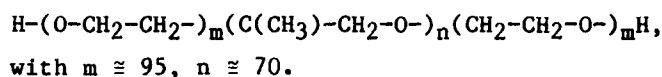
maximally. The resultant water-transmission reduction of only 50 percent by the foam is rather unimpressive; measurements of the foam in vivo are needed to be able to rank this method of covering (1,11).

The wafer material, already in use ("Stomahesive") for ostomy-bag attachment, consists of a mixture of gelatin, pectin, sodium carboxymethyl cellulose, and polyisobutylene. It adheres readily even to moist surfaces, but can be removed for examination of the wound site without disturbing advancing epithelium (96). Water-loss characteristics of this material are unknown; but it has been used as a protection for healing skin ulcers, and would presumably serve for mild burns or donor sites.

2.4 Pluronic F-127 Gel

This material, which has not been described in the literature since 1972, is included because of its unusual gelling properties. It forms a gel at about 20-90 percent concentration in water solution at room temperature. At slightly higher temperatures, it gels at a lower percent solution in water, e.g., at 25°C it forms a soft gel at 18 percent, and a hard gel at 20 percent. At lower temperatures, higher concentrations gel less. Thus an interesting application involves making a 20% solution in cold water, then heating to skin temperature by applying it to a burn, inducing gelation. For use as an immediate wipe-on for burns, the initial liquid solution could contain alcohols (including glycols for eventual flexibility control) and bactericides, silver nitrate and silver lactate having been used in animal tests. Obviously any other soluble or suspendable agent could be used in the same manner, with possible slight perturbation of the gelling behavior. This material is clear as a gel and would permit visual assessment of underlying wound healing. It would be removable by bathing.

Chemically this material consists of a poly(oxypropylene) core, grafted on each end with poly(ethylene oxide). Graphically



for this particular member of the Pluronics series. The water transmission characteristics of this polymer are unknown; its adhesion in animal trials was adequate (68,69). It is not reported how it adheres to an oozing-type burn surface; it might well dissolve (a problem common to all these water-soluble uncrosslinked gels).

2.5 Poly(hydroxyethyl methacrylate) (Hydron)

Poly(hydroxyethyl methacrylate), or p(HEMA) for short, is a material of considerable current interest. This derives from the considerable biocompatibility of this material, not only as a polymer, but also as residual monomer (227). The single hydroxyl group on the side chain enables gels of a wide range of solvent contents, and structural strengths, to be made; also, it gives the hydrogel a low interfacial tension which results in less protein absorption, and greater diffusibility to small molecules (200). In addition, some sticky cell types will not adhere, e.g., PMN leukocytes (164).

In its clinical use as Hydron Burn Bandage the p(HEMA) is dusted onto the wound alternately with a spray coating of polyethylene glycol (PEG 400), building up several layers. The PEG dissolves the p(HEMA), resulting in an even coating of p(HEMA) goo. (The polymer, even as a 5% solution, is too tacky to wipe on as a liquid, hence the need for formation in place). The PEG is gradually absorbed into the burn, and excreted within about 24 hours; it is known to be quite nontoxic (228). Although it then becomes partially hydrated, the resultant Hydron layer is permeable enough to water (estimated pore size 4-5 Å) (200) that the eschar underneath remains in a state described as "dry". At the same time, there is alleged to be a reduction of water transport over that of uncovered eschar (196). (No data on this have been reported for in vivo use, however.) There would appear to be a fundamental opposition between keeping the burn site moist to

prevent dehydration and further necrosis, and letting the eschar dry to facilitate excision or spontaneous separation. We believe that optimal treatment by a universal immediate covering should keep the site as moist as possible and allow penetration of antibacterials.

It is believed that the slightly beneficial results of Hydron (over uncovered controls) seen in animal tests derive from its inability to support bacterial growth. It is essentially non-biodegradable, and may inhibit bacterial growth slightly by initial hygroscopicity when applied (20,180,196).

Advantages of Hydron for human use include reasonable permeability to antiseptics (Sulfamylon, Silvadene) (184,196), although they could also be included in the initial coating material. It affords pain relief, a check on bacterial growth, and less dressing changing than with silver sulfadiazine cream. However, some major disadvantages exist, namely that the dressing is difficult and time-consuming to apply, adheres poorly in the first 24 hours (often not at all to moist second degree burns), requires (in 1/3 of a clinical study's cases) repair of cracks and fissures, and obscures the wound, hence must be snipped away for taking bacterial samples. In addition, it softens and tends to roll up and off at the edges on exposure to water (30). These factors led to Hydron being recommended for first and moderate second (up to 20% BSA) burns and donor sites, but non-recommended (vs. Silvadene) for any worse burns. These disadvantages render this material far from ideal as an immediate post-burn covering.

2.6 Other Gel Types

Several other types of gels, and some currently marketed products, exist. Use of two former types of materials, polyacrylamide gels and polyelectrolyte complex gels, as burn coverings has not been reported on recently (141,187,200). Of these two, the mixed ionic gels (brand name Ioplex 101) were of interest because, although they were extremely permeable (up to $MW \approx 10,000$) (hence probably mediocre at water retention), they were

flexible, resilient, and adhered strongly to tissue (in fact, they were considered as sealants around percutaneous electrode wires). Considerable work was done to develop systems with controlled gelation times, using a prepolymerized anionic material, a cationic monomer, and redox initiators. Some systems gelled with these components only; another type required addition of aluminum salts. In addition, some protection from air oxidation was desirable (141). These materials, it would seem, would be useful as a two-part spray-on film; however further published work was not done, although the materials were investigated for use as a disguise-type adherent skin-covering masks.*

Other gel-type materials which have appeared recently include three. First, a carbohydrate-based gel (of plant origin) has been incorporated in a fire blanket on the market, for essentially temporary protective use during building fires, but secondarily, also for first-aid use on burn victims. Use as first-aid is overshadowed by claims that the blanket, by slow evaporative action, affords better thermal protection than essentially insulative suits (143).

Second, a simple gel material (supported by an internal plastic mesh), a substituted, crosslinked, poly(oxyethylene) or poly(oxypropylene) gel (brand name "Second Skin"), is sold for use on minor burns, abrasions, and other skin irritations (121,122). Its adherence qualities are only those of a wet gel; for better water retention one leaves an outer plastic backing layer on during application.

Third, a dextran gel, crosslinked with epichlorohydrin and reinforced with cotton gauze, has been hailed as a burn covering. Its advantages are tackiness to moist tissue, permeability to penicillin, non-immunogenicity, and low cost (36).

* Tom Rich, Research Division, Amicon Corporation, 25 Hartwell Avenue, Lexington, Massachusetts 02173. Personal communication.

2.7 Cyanoacrylate Adhesives

At the extreme end of the covering spectrum, we find materials which were of interest for their extreme adhesive properties, the n-alkyl alpha-cyano acrylates. Although the butyl and higher members of the series are well tolerated by tissues, these materials have not been reported as burn coverings for some time (176,178), probably because of inadequate vapor permeation control. In addition, these materials, which are stored and used as monomer and polymerize in situ, are not stable for long-term storage.

SECTION 3

POST-EXCISION BURN WOUND COVERINGS

3.1 Introduction

In the preceding section, potential candidate materials for an immediate post-burn covering were reviewed. None of these materials, however, is intended for prolonged use on an extensive, severe burn, for essentially two reasons: one, because the dead tissue (eschar) cannot be absorbed by the patient before it is colonized by bacteria, and two, because such an inflamed, liquid mess would not support wound closure. Therefore, at some point, the temporary covering must be removed, the eschar surgically (or otherwise) removed, and the wound closed by some sort of post-excision covering. The optimal material for this (assuming an uninfected wound) is autograft, as has been known for a long time. The most modern technique involves meshing and spreading autograft 6:1, then covering with (frozen-stored) allograft (cadaver skin) meshed 3:1 (to offer mechanical protection), and wrapping to absorb exudate. Both graft materials initially adhere to the wound; after 3-30 days (median 14 days), the allograft is rejected, but the autograft continues to spread to cover the site (257). Although this technique allows for much less donor site exposure than did the former use of unexpanded autograft, still, because of trauma to the patient, surgical prudence dictates as of now that no more than 25% BSA total be debrided or used as a donor site, in any one procedure. Thus no more than about 20% BSA may be debrided at once, by this technique. This limitation is set by blood losses during surgery, time of surgery, and fluid losses through the exposed areas post-surgery. However, another limitation that may arise is the lack of enough healthy donor-site skin to resurface the burned areas. In such a case, the need arises for a suitable skin-like post-excision covering.

The properties necessary in such a covering are listed below, in approximate order of importance:

Sine-qua-nons

- 1) Adherence
- 2) Barrier to bacteria (total) and water (partial)

Extremely important

- 3) Non-antigenic, non-allergenic
- 4) Elastic and flexible, but strong enough to be secured as necessary
- 5) Available

Rather important

- 6) Sterilizable
- 7) Capable of indicating infection beneath
- 8) Not easily bacterially degradable
- 9) Minimizing of scar formation

Useful

- 10) Incorporable by the body, at a controllable rate
- 11) Accelerative of wound closure
- 12) Allowing control of infection without removal; implies permeability to anti-bacterials
- 13) Restorative of normal skin function
- 14) Inexpensive

The ideal covering will then carefully regulate the environment of the wound site to permit, or even accelerate, eventual formation of normally structured skin. In addition, general systemic benefits enjoyed by the patient over less ideal coverings might include reduction of the usual heat losses due to water evaporation at an open burn site (270); possible reduction of metabolism to more nearly normal levels, and earlier resumption of mobility. The state of the art is still far from such a single covering, or method of treatment, even though significant advances have been made recently.

The materials which have appeared in the literature since 1977 will be discussed in this section in approximate order of technological (and chronological) development. Thus logical groupings include: A) skins (cadaver and animal), B) collagen materials, C) purely synthetic materials, D) hybrids of collagen and synthetics, and E) cell-cultured human skins.

3.2 Skins

3.2.1 Introduction

Skins for burn coverage bear a certain resemblance to food: they are best when fresh, a little poorer when frozen, and have definitely lost a little something when freeze dried. However, in practice, economics and supply may force use of the second rate products. Currently in use as skins we find various human products, and porcine skins.

3.2.2 Human Skins

Human products of recent note fall into the categories of cadaver skin, amnion, and dura mater. Cadaver skin, though retained longer as a graft than porcine skins, is eventually rejected because of antigenic mismatch. Immunosuppression, formerly practised to delay this eventuality, is inadvisable because of the already poor immune responses of burn patients. There is a lack of viable fresh cadaver skin because, even at 4°C storage, cadaver skin remains viable for only approximately two days (23,24, 25). (It is interesting that under the same conditions, autograft lasts only one day, as measured by a glucose-metabolizing assay.) Therefore, cadaver skin is more usually frozen or lyophilized. One report (138) found freeze-drying to diminish the immunogenicity of skin, presumably by some kind of structural damage. The effect was abolished by protection with poly(N-vinyl pyrrolidone) (PVP) during freezing. Cadaver skin is usually considered too expensive to qualify as an ideal post-excision covering, in addition to being eventually rejected.

Amnion, because it contains an epithelial layer and therefore somewhat models skin, has been of interest for some time. It may be stored alive in tissue culture for up to three weeks (98), or used dead from storage in disinfectant solutions, or in saline after disinfection. Amnion, because of its thinness, disintegrates readily on the burn wound; therefore it must be changed every two days or so. Even so, it retains fluid to some extent, diminishes pain, and allows reepithelialization across a granulation bed. Though this use cannot be particularly recommended, it has been used to strip eschar from second and third degree burns in children (10,75,81, 208,243,251). Although amnion is reasonably available (being storable for up to eight weeks in penicillin solution) and inexpensive, its relatively high moisture loss rate, and impermanence, make it rather unsuited to be called an ideal post-excision covering.

One mention was made of a trial of human lyophilized dura mater ("Lyodura") as a epithelial replacement in hamster cheek pouches. It cannot compete with even cadaver skin in effects or availability (90).

3.2.3. Porcine Skins

Because of the limitations on human skins, porcine skin has been widely used, and is generally what is referred to when the term "biological dressings" is mentioned. The use of porcine skin has been recently reviewed (73,78). Porcine skin, or "heterograft" (to humans, anyway), because of basic similarity to human skin, is superior in its retention of water at the burn wound (83). However, even though it exhibits adequate initial adherence, it eventually is rejected. It is recommended to be changed every 2-3 days, if the wound is clean, as it will be rejected in 4-5 days. An interesting modification, glutaraldehyde-treated pig skin, is said to be retained 8-10 days by comparison (43), presumably due to diminution of antigenicity and decrease in digestibility by local enzymes (bacterial and human) caused by crosslinking. It was even said to resist maceration on an infected wound, a gruesome trait. Pig skin is often lyophilized for convenience and supply, with attendant problems of reconstitution time before use. Finally, there appears to be a small (but hotly

debated) possibility of vascularization if left on too long on a donor site (73). All these factors, plus the realization that biological dressings are no longer as superior to synthetics for control of infection as they once were, has led to the following limited recommendations: use it for protection of granulation tissue (it will adhere but not vascularize), or for debridement, only (even though it is somewhat expensive for these purposes). Thus porcine skin (split-thickness epidermal layer, or Corethium 1) is also far from an ideal post-excision covering.

3.3 Collagen Materials

Collagen has been long known as a promising material for an artificial skin. As such it has enjoyed extensive research and formulation into a multitude of forms (10). Perhaps its most salient advantage over other materials is its weak antigenicity, which can be further reduced and altered by various treatments. As a transition stage from the skins above to modified collagens, there is available a material "Corethium 2", nothing more than the second slice taken by dermatome from a pig, after the first slice has removed the epidermis. This may be lyophilized, etc., just the same as epidermis, and has seen some recent use as cover for ulcers, burns, and donor sites, particularly in Britain (49,74,80,94,118,204). Despite what one would assume to be inadequate moisture retention, it nonetheless has functioned well in these trials in general, but may be somewhat inferior to synthetics in bacterial control. Similar earlier products, for example a porcine collagen obtained by trypsinizing whole skin, were suggested to be of possible benefit in preserving the morphology of the skin. However, hopes that this intact, mature collagen would be better at promoting adhesion (276) or diminishing contraction seem to have fallen into disrepute, in favor of more altered forms of collagen.

Collagen films and sponges appear recently in the literature (8,10). However collagen films alone (Cutycol® is a common brand) are not very infection-resisting, and they have mechanical problems, specifically,

shrinkage which leads to sliding and nonadherence for large sites (95,191). In addition, this shrinkage makes them unsuitable for circumferential sites. Thus collagen films, by themselves, are no longer considered for burn coverage. However, collagen may also be fabricated as a sponge, with adequate mechanical stability when crosslinked by glutaraldehyde, hexamethylene diisocyanate, or the like (57,163,167,210,258). Collagen in this form exhibits adequate, even noteworthy adherence and conformability. It can be used to debride burns, though as an eventual covering it leads to more contraction than Epigard, a similar form of synthetic product. A foamed collagen could incorporate a variety of different additives; one early material incorporated reduced silver as an antibacterial. This field has been reviewed with respect to burn coverings (57,183,197,198), though not recently.

3.4 Synthetics

3.4.1 Introduction

One route to avoiding the high costs of biological coverings, and avoiding the technological problems of fabricating collagens, has been to turn to the use of synthetic materials as post-excision coverings. Synthetics are often provided with a textured undersurface, often an open-cell foam, for use next to the wound; this provides for adhesion due to ingrowth of collagen fibers. In addition, some top layer to allow only the proper small water loss is necessary. In this category the polyurethane, poly(ϵ -caprolactone), and Silastic foams fall. Other approaches to wound coverage include single or multiple component films and surface-grafted polymer materials. These types will be considered in this order.

3.4.2. Foam-Based Coverings

Of foams, the most used is the commercially available polyurethane-based material Epigard® (74,76,120,186,234,237). This flexible covering, ca. 1 mm thick, exhibits many desirable properties: pain reduction, good adherence and also removability, low cost, easy storage, autoclavability,

and ease of application. It may vascularize, which should be avoided by occasional replacement; it is strong enough not to fragment on removal (as was a problem with the precursor poly(vinylalcohol) foam, "Ivalon," which, incidentally, is still being used in studies (74,242,248,249).

The water retention is provided by a top-layer of micro-porous Teflon (formerly of polypropylene) (166). Epigard may be used to cleanse wounds (by repeated changing); once wounds are clean, users declaim its trouble-free action (88). Its two reported disadvantages are the need for occasional replacement, and a tendency to adhere so tightly that it may delay epithelialization of superficial lesions (120). Indeed, it is difficult to see how epithelium would not grow into the sponge, only to be ripped off upon dressing replacement. Thus Epigard, and its twin material, a polyurethane foam/polyurethane film (92), as well as its sister, Lyofoam (10,65,117), narrowly miss being ideal post-excision coverings.

In the same line, a poly(ϵ -caprolactone) foam, bonded to a similar film, was investigated. Infiltration by tissue, leading to partial retention (on rats) upon removal on the tenth day post application, indicate that this material, too, would need to be periodically replaced to avoid vascularization. Its mechanical properties and water-vapor transmission rate (1-2 g/hr-cm²) were all quite acceptable (158).

Lastly, a larger pore silicone Silastic based foam has been tried out, both as a burn covering (38,66), and as an air-permeable space-filling stent for surgical defect wounds. For the purpose of keeping water in, it may be in fact too impermeable as a small-cell foam; however when used as a wound cover it appears to be unsuitable by reason of nonadherence (33,47).

3.4.3 Films

Under certain circumstances, single-component films find use in covering wounds. Op-site®, a polyurethane, moisture permeable film supplied with an adhesive which bonds only to dry skin, is a prominent material in

this regard. Its primary use is as sutureless surgical closure (72,159, 268), for which it is admirably suited; however, it has also been used to cover graft donor sites and second degree burns (71). Drawbacks include limited water transport, often necessitating puncture or exchange during the first 2 days post-operatively to release accumulated fluid. Although this fluid may appear purulent, this does not betoken clinical infection. After this, however, it may remain in place until it separates spontaneously, leaving healed skin beneath (64,95). There is no need to worry about tissue ingrowth, since the pores of the Op-site are too small to support this. Thus, Op-site, by virtue of its limited water-vapor permeability, does not qualify as an ideal post-excision covering.

In an attempt to provide a film with proper permeability and versatility, a copolymer of dimethylaminoethylmethacrylate (DMAEMA) and acetonitrile was investigated (60). This system permitted incorporation of plasticizers and antibacterials (although the release properties were not studied in detail), and allowed the water-vapor transmission under estimated wound conditions to be controlled over the range of 2-6 mg/hr-cm². This control was effected by a post-fabrication step of crosslinking with ethylene oxide. This material, although physically interesting, has yet to be proven in animal tests; in particular, its adherence, which may be affected by the charge of the dimethylaminoethyl side group, has yet to be established.

In a different line, the possibilities of a post-excision type burn covering made by radiation-grafting HEMA (hydroxyethylmethacrylate) to a Silastic sheet, were investigated. The essential purpose of this approach is to provide a minute layer of HEMA, whose adherence to burn sites (as a gel) is already known (though not spectacular), and whose non-inflammatory properties are well known, intimately bonded to the structural and water-retaining Silastic. HEMA as the primary graft material offers some advantages, in that its water content and hydrophilicity can be controlled by the grafting conditions and by the use of additives such as ethyleneglycol dimethacrylate (EGDMA) and ethylmethacrylate (EMA) (55,133). In general, wettable grafts permitted slightly higher water passage than did

non-wettable grafts, due to changes in the porosity of the grafted layer. In no event, however, did the HEMA layer alter the base transmission of the Silastic film (about 1 mg/hr-cm² for a .13 mm thick film) very much. This is a very low transmission rate, and thinner base films would appear necessary to achieve acceptable permeability. These might be too delicate to handle, unless backed by some inert exterior material. An interesting effect is that the more hydrophobic additives above led to a more wettable surface, by sequestering the hydrophobic portions of the HEMA molecules in the interior of the graft layer. A related material, HEMA grafted onto polybutadiene, has also been looked at (19).

The question of adhesion is an interesting one in this connection. Although the above researchers believed that the wettable surfaces would be more desirable for a covering, this is by no means apparent. Simple pretreatment of a wet tissue surface with benzoyl chloride, for example, will benzoylate active amine groups (as on lysine) and result in non-polar adherence of materials such as hydrophobic adhesives (14). Certain cell types, such as mouse fibroblasts (3T3), adhere better to hydrophobic-type surfaces (56). Thus, it is by no means certain that slapping a hydrophilic surface onto the wound is the way to achieve optimal adherence.

Another possibility for causing adhesion lies in activating the dressing surface so that it would chemically, or enzymatically, form links to the tissue below (31). The technology exists for specifically attaching proteins (or other molecules) to surfaces such as HEMA (225), so that they could activate the normal attachment scheme (to fibrin/fibronectin). In fact, a material in the next (hybrids) subsection may have done just this.

3.5 Hybrids of Collagen and Synthetics

Because of the resorbability of collagen, and the barrier properties of synthetics, there has been some impetus towards laminating these two materials into a bilayer covering. For example, one combination investigated involved sausage-casing collagen with a poly(ϵ -caprolactone) film overlayer. This material adhered moderately well in rats (158).

Two materials of current use exist. The first, Biobrane® (21,156) (Woodroof Laboratories), consists of a silicone sheet overlayer into which a nylon velour is cast (on one side). On the textured side, a further coating of a collagen derivative has been cast exceedingly thinly (so that the loop structure of the nylon is completely unobstructed). This product is recommended for use on donor sites, superficial second degree burns (without debridement), excised second and third degree burns, and over meshed autograft. It has been reported to remain intact and adherent over infected sites, but removal for treatment in such cases should not be difficult. It is also somewhat permeable to silver sulfadiazine. The only drawback to this material, in fact, is that, due to the nylon loop structure, separation of the material as reepithelialization occurs might not occur. This point has not been reported on in the literature, although velour-underside fabrics have been previously investigated (10,14,226).

The second material (as yet unnamed), created by I. V. Yannas, at M.I.T., which has undergone clinical trials and is reported to be on the verge of commercial production, is a three-material composite of collagen fleece (a very open foam) intimately admixed and crosslinked to a glycosaminoglycan, chondroitin-6-sulfate, with an outer layer of silicone rubber sheet. The glycosaminoglycan is understood to render the collagen totally non-antigenic by its presence, and the crosslinking (accomplished with glutaraldehyde and/or dehydrothermally) to control the rate of breakdown and resorption at the wound site. Due to this control of resorption, good clinical results have been achieved where the membrane served as a covering for up to 50 days (20 is median for cadaver skin, and 6 for pig skin, before they are rejected), with the limitation being not rejection but rather contraction on small to medium-sized wounds.

As this report went to press, a published report detailed a "stage two" of this material. In this modification, basal epidermal cells are isolated (but not tissue-cultured) from a skin specimen of the patient and seeded into the collagen/GAG fleece by centrifugation. In animal trials, this resulted in rapid epidermal growth from within the membrane;

results from use of this stage two membrane on humans (which would eliminate the need for even the epidermal autograft) are not yet reported.

An optimal moisture flux of $0.5 \text{ mg/cm}^2 \text{ hr}$ is claimed; that this is much lower than optima found for other coverings may be an indication that this dressing provides a much less inflamed wound site, by its (intended) similarity to natural dermal composition (2, 3, 4, 6, 7, 123, 124, 278, 283).

This dressing, then, is the first* to provide the property of incorporability by the body at controllable rates, a significant advance (the silicone sheet peels off as it is undergrown by advancing epidermis). Such a collagen-containing covering, of course, might have additives included for purposes of additional scar minimization, bacterial reduction, or growth control. Use of such additives in the covering has not been reported, nor has the amount of residual scarring from this treatment. It also remains to be seen how expensive the final product will be; its manufacture requires considerable manipulation of ingredients.

One final problem that has not been addressed up to now concerns the replacement in the burn area of sweating capacity. Normal pervaporability to water scarcely serves to cool skin, and heat intolerance of burn victims (after complete recovery) is a permanent residual effect whenever large full-thickness burns have been sustained (61). Possibly this future goal might be achieved through tissue cultured skins, the next, and last category of covering.

*Although a 1970 German patent for a related mucopolysaccharide metal-tanned-collagen-sponge multilayer material exists (183).

3.6 Tissue Cultured Skins

3.6.1 Introduction

Recent advances in the technology of in vitro tissue culturing have made possible to approach an answer to the question, "What if we could grow skin for the burn patient, and then use it on him?" This would be truly the definitive closure method, a biologically totally harmonious route. Recently published research of three groups working on this problem, with somewhat different techniques, indicates that it is only a matter of a few years before in vitro grown skins will be available for burn wound (or other skin loss) problems. The primary problem with these materials that needs yet to be surmounted, is that of time, with typical needs of 14-22 days to grow $\approx 1 \text{ m}^2$ from a 1 cm^2 specimen. Let us consider these materials and the details of their fabrication closely. I shall designate the materials by the names of their senior investigators, as Bell's, Eisinger's, and Green's, respectively.

3.6.2 Bell's "Skin Equivalent"

Bell's approach (62,70) has been to try to create a full thickness (epidermis and dermis) material, thus enjoying the water and bacterial barrier properties of an epidermis along with the contraction reduction and structural integrity of a dermis. From the initial skin specimen, he therefore would ultimately need to obtain both the fibroblasts and the basal cell epidermal cells; at present he collects the fibroblasts which migrate from one specimen and obtains epidermal cells from another. The fibroblasts are grown in culture to confluence, and recultured as necessary to obtain $\approx 5 \cdot 10^4$ cells/cm² of eventually desired "skin equivalent" final product. The cells are removed (removal and dispersion is done by trypsinization throughout), suspended with acid-swollen collagen and serum, and the lot coprecipitated by neutralization. This loose collagen and fibroblast mat is then actively contracted by the fibroblasts, an undisclosed portion of which acquire myofibroblast character, resulting in an undisclosed

smaller, firm matrix. This material is washed, seeded with an epidermal suspension, and maintained in culture; the epidermal cells grow to confluence, and the material is ready for use. Some problems with the material, up to this point, are these: first, time involved - adding the given minima of 4 days for fibroblast migration from the biopsy, 4 days for initial culture, 10 days to grow in secondary culture (enough for a 100 cm² product), 4 days to contract the lattice, and 2 days to grow epidermis to confluence on it, yields 24 days minimum to make this material in quantity. Secondly, for the final growth stages, Bell says he needs homologous serum, either autologous or close, carefully typed. This may not be so easily obtained.

Bell's material has not been yet reported in human trials, but on rats, the following observations may be of interest: 1) The graft vascularizes in about 7 days, and grows in thickness thereafter, with remodeling of the collagen. Though redness gradually diminishes, a scaly/dry character, due to lack of sweat/sebaceous glands, remains. 2) By 10 weeks, a normal dermal collagen (basket-weave) pattern can be seen at the edges of the graft. It is unknown whether the whole graft eventually converts to the normal pattern; the whole graft does however exhibit increasingly birefringent collagen (an indicator of normal, mature type I collagen). 3) By 10 weeks, the dermis of the graft is still only about half the thickness of normal dermis (1.3 mm). The graft dermis appears compact. Thus, exuberant collagen formation is avoided by use of this covering. Certain other minor structural irregularities remain in the skin, besides the lack of adnexal structures (Bell admits the need to recover, propagate, and try to incorporate these other cell types. Achieving their differentiation and functioning in this context, though possible, remains quite a ways in the future.)

3.6.3 Eisinger's Epidermis

The approach taken by Eisinger (154,155)(and Green, in the next section) is to cultivate only an epidermal cell layer. For handleability,

this needs to be somewhat thicker than just a barely confluent layer as is the case with Bell's skin; 11-13 layers of cells, achieved in 15-20 days, is adequate.

Eisinger has studied the histology and metabolic character of the cells in her materials quite thoroughly, and has found several varieties to be present in her materials as they grow. In general, the growth of her material can be characterized as 1) basal cell multiplication, then 2) a wave of differentiation.

This material is grown directly on tissue culture plastic (as are they all); growth on a collagen film (Helitrex) was found possible but slow (3-4 layers in 21 days!). If initial seeding is greater than $\approx 1 \cdot 10^5$ cells/cm², preferably $2.5 \cdot 10^5$ cells/cm², the pH 5.6-5.8, and the temperature 35-37°C, the cells will grow to confluence and form the desired cell layer (if not, discrete colonies are the result).

When ready for use, the membrane is transferred via a collagen over-film pick-up. This material has also only been reported on in animal tests.

3.6.4 Green's Epidermis

Green's material (150,151,152), also an epithelial layer only, differs only in technique of cultivation from Eisinger's. Rather than tissue flask, Green uses a lethally irradiated 3T3 (fibroblast) layer as cultivation surface. He also uses chemicals to increase growth rate and vigor of the cell cultures. These materials are two: EGF (epidermal growth factor) to increase cell mobility, and cholera toxin, to improve the growth rate of the cells. Thus he is able to deliver, from 1 cm² biopsy, 1500 cm² of epithelium in 14-19 days, with an overall cell increase of some 500x during this time.

Some interesting observations on his material are these: 1) on mice, the covering did not particularly stop contraction (a 2x2 cm wound, so covered, ended up 1.5 x 0.3 cm!). Nonetheless, in human trials (2 patients) the culture-grafted areas, vs. meshed split-thickness-autograft-covered areas, were after 5 months "no more contracted or fragile." The grafts were not notably adherent: "over 50%" showed initial take over freshly excised areas, and the primary cause of graft failure was thought to be infection.

Green estimates that he could grow an entire body-worth of epithelium in ≈ 5 weeks from an initial 1 cm² biopsy. This is still quite a time period; he suggests that the method might find some utility in growing cultured allograft for temporary coverage for more immediate use.

One additional problem with his (thin) epidermal layer (apparently less with Eisinger's thicker one) is its shrinkage upon removal from the culture dish, to $\approx 1/4$ its original area. This would appear to necessitate very large culture dishes in order to have conveniently sized pieces for use at the operating table.

What advantages do these three materials offer over other types of coverings? First, they offer guaranteed biocompatibility, in the case of a covering using only cells derived from the patient. This might be expected to allow more exact control of contraction than an inert, resorbable covering could allow. Second, they are available in almost unlimited supply, although only after a considerable induction period. Third, they promise eventual control of the dermal thickness and morphology, with (hopefully) intact hair and glands. Although expanded split-thickness autograft appears to provide eventual acceptable pigmentation, and also satisfies point (1) above, it is deficient in point (2) and fails on point (3) altogether. Other coverings, such as Yannas', which appear to satisfy point (1) and (being fabricated) point (2), also fail point (3). Hence, one must conclude that tissue cultured skins show definite promise as future post-excision burn coverings.

Section 4

GROWTH FACTORS

4.1 Introduction

In considering the role and possible incorporation of "growth factors" into burn wound coverings, it is important to consider what it is that we wish to do. In the broadest sense, a "growth factor" is any substance (thus excluding treatment modes such as light (214) or electrical current (273)), which will contribute to regrowth of the skin over the burn wound. However, this classification would include any material at all with an effect on the total burn healing process. Most observers would concur that chemicals specifically acting to reduce inflammation, to inactivate necrotic processes at the wound, or having broad systemic effects but not local ones, would not be considered growth factors: a growth factor must cause increased growth of cells or of tissue (antibacterials, another major candidate for use, are treated in Section 5). For use in a burn covering, a growth factor must be better applied topically than systemically. It might, for example, only exert an effect locally at a burn site, and still be more advantageously administered intravenously. Since use of "growth factors" in healing burns is still quite uninvestigated (up until very recently no adequate burn covering was even available), there is obvious need for eventual comparison of the effects in burn repair of these materials administered topically vs. intravenously; however, for the present we could choose among several materials for an initial topical evaluation.

To introduce the materials, first consider which target cells might be stimulated at the burn site. One group, those of the immune system, we will explicitly exclude for the following reason: the immune system has total-body problems which will not be solved by locally attracting leukocytes to the wound, or inducing them to stay. (Biochemicals for the above two purposes, though researched somewhat, would hardly be cost-effective at present). Rather the burn-induced immunosuppression must eventually be treated systemically, as mentioned in Section 1.6.3. This

leaves the categories of endothelium (needed to multiply and vascularize new tissue), fibroblasts (necessary to synthesize the dermal matrix of collagen, glycosaminoglycens, and possibly fibronectin), and epithelium (necessary to provide barrier properties) as possible target systems for chemical trickery.

What, in a general way, can one do for these cells? It may be that a limiting material is a nutrient: although regular alimentation needs of burn victims have been studied for a long time, local topical supplementation might have a minor beneficial effect. Falling into this category might be accelerators such as poly(N-acetyl glucosamine) (169), cis-hydroxyproline (185), arginine, pyruvate, and putrescein (212). Alternatively, one could perk the cells up with a 'shot in the arm' booster of topical ATP (238). Another quite specific possibility is treatment of specific enzymes to increase their activity. Falling into this category would be such substances as prolyl hydroxylase inhibitors or effectors, anaesthetics (101), cross-linking controllers (lathyrogens), corticosteroids (182,231), prostaglandins (146,212), hexadecane (271), thyroid hormones, and retinoic acid.

By technical biological usage, however, growth factors are none of the above; rather they are specific biochemicals which give their target cells a tremendous metabolic boost, and can be best thought of as specific substances used to transmit "turn-on" signals to tissues (i.e., super-hormones). Three of these (of burn healing interest) have been identified: EGF, FGF, and TAF. These proteins, it is thought, act by causing changes in the adenyl cyclase activity of target cells, resulting in changes in the cells' cAMP and cGMP contents. These last two substances then act further within the cell to make the cell synthesize products, divide, or whatever its target action may be. (The mechanics (160) are not of concern here.) In addition to these specific growth substances, other chemicals can have similar action on the adenyl cyclase enzyme, and thus similar results. Let us consider first the growth factors, and then such chemicals, in turn.

4.2 Epidermal Growth Factor (EGF)

This single chain, 53 residue polypeptide has been studied the most extensively of the three growth factors treated here. All mammalian EGFs are close in structure and exert the same effect, therefore EGF from mouse submaxillary salivary glands (once known as nerve growth factor, NGF) is usually used for research (279,280). In general the gross effect of EGF is to prevent differentiation, increase mitosis and especially to increase the motility of epithelium. This is true in rabbits and humans (35,84), mice (97,151,154,215) and presumably also pigs. In tissue culture, EGF acts similarly on bovine endothelium; one report speaks of a "more orderly" vascularization of a wound (35). Concentrations of 5-20 mg/ml (272) in tissue culture, or 17 µg/oz (35) in salve, is sufficient to exert these effects. This material acts only as long as supplied to the target cells. (Used in cell cultures and necessary at high cell densities, it can even cause cells to eventually become dependent on it for mitosis (215).) Therefore, since it is a protein, and the burn site has considerable protease activity, one must consider the likelihood of its destruction before it can act on the epidermis.

In addition to this, there is a considerable caution in this field about extrapolating results from tissue culture, to organ culture and in vivo situations. Frequently, a specific effect is found to disappear or even reverse, when the substrate changes. (As an example, bovine corneal epithelial cells, in tissue culture, will not mitose in response to EGF unless they can make a basement membrane, which they refuse to do unless provided with a collagen substrate. However, FGF is effective in inducing their mitosis, regardless of their substrate (84).) Lastly, we consider the effect of EGF on fibroblasts: although earlier reports were of mitogenesis, in a topically salved rabbit-ear wound, fibroblasts oriented themselves, and produced a more mature collagen faster, with no report of increased numbers. In this study, total wound closure time was unchanged: lessened contraction balanced faster epidermal migration. This is exactly the effect one would like to produce, to reduce scar formation.

Overall, EGF is a not unpromising candidate for a covering-incorporated growth-factor; it has unique effects on epidermal spreading*.

4.3 Fibroblast Growth Factor (FGF)

This material is believed to be formed from breakdown of myelin base protein, perhaps by cathepsin D (77). It is a polypeptide of about 90 bases. Its greatest proven effects are on myoblast mitosis, which it effects potently and immediately. It has certain reported epidermal effects (84,215) but primarily considerable endothelial growth power. It has been implicated in experiments on limb regeneration (77,273). Specific effects on fibroblasts are unknown. This is a possible material for use in a covering.

4.4 Tumor Angiogenesis Factor (TAF)

This substance, so called after its production by tumors to attract blood vessel ingrowth, is known to stimulate (besides angiogenesis) human fibroblasts, for which it co-requires a collagen stratum and a platelet factor (58,59). Sustained release of TAF from a biological implant has been demonstrated (5,256).

4.5 Calcitonin

This hormone (salmon calcitonin is least degradable and thus most effective), given systemically, stimulates epidermal cell activity in rabbits, including higher rates of protein synthesis, keratogenesis, and collagen formation by fibroblasts, and leads to accelerated wound repair (115). Since 0.2 mg was an effective dosage/rabbit, one would have to classify this as fairly potent and possibly deserving of the name growth factor. It is a possibility for trial in an immediate post-burn covering.

*Phorbol esters share some biological effects with EGF; it is not clear if epidermal spreading is one (272).

4.6 Miscellaneous Biological Growth Interaction Materials

Certain other substances have been recently reported, with undetermined potencies. These are 1) a material produced by fibroblasts with a positive effect on epithelial multiplication (215), 2) a platelet factor (MW \approx 13,100), which, in combination with thrombin and another, unisolated platelet component, stimulates fibroblasts and endothelial cells (99); 3) an antibody (deliberately created) to fibroblast-cell-surface fibronectin, which causes much mitosis and somewhat increased synthesis of GAG by the treated fibroblast (147); 4) a material produced by macrophages, which activates keratocytes (85); and 5) (a) small polypeptide(s) from partially hydrolysed casein, which stimulates prepuce cells in culture (277). Too little is known of these materials to make them candidates for incorporation in a trial covering.

4.7 cAMP, cGMP

These are the two intercellular materials which are thought to be involved in the effects of the growth factors, particularly EGF. Therefore, one might wonder if they can be absorbed topically into target cells. In tissue culture, 10^{-4} M cGMP raised epidermal mitosis by about 60 percent (262); interperitoneal cGMP in excised rats, caused less intensive inflammation, faster epithelialization, thinner granulation tissue, and a thinner, more compact eventual dermis. Peak number of fibroblasts was unchanged but attained earlier during the course of healing. A similar effect, seen earlier (119), caused by an extract of actively growing organs, and thought to be caused by cyclic nucleotides, was more effectively exerted interperitoneally than topically.

4.8 Adenyl Cyclase Effectors

Various materials exert effects on adenyl cyclase, thereby altering cAMP and cGMP in target cells. Here are a few that have been studied.

4.8.1 Cholera Toxin

This toxin, at incredibly low levels, (10^{-9} - 10^{-11} M), irreversibly activates adenylyl cyclase. In human epidermal cell cultures, this means that the epidermis is immediately and drastically induced into mitosis. Because this effect is "irreversible", that is, continues for at least two tissue culture transfers, this material has a great potential for incorporation into a wipe-on, since one application would stimulate for days, even after the protein was lysed. However, the toxin alone does not increase epidermal spreading, at least in tissue culture; EGF is necessary for this (215).

4.8.2 Dibutyryl cAMP, Methyl Isobutyl Xanthine, Isoproterenol

These three compounds (besides exerting other effects severally), all increase cAMP, and also epidermal cell growth in culture (212,215). Optimal concentrations were on the order of 10^{-4} , 10^{-4} , and 10^{-5} M, respectively.

4.8.3 Histamine, Epinephrine

Histamine, by H_2 -receptor action (partially reversible with cimetidine) at 10^{-4} M inhibits porcine epidermal mitosis; by H_1 -receptor action (not drug-alterable), at 10^{-3} M it partially negates the H_2 -receptor effect. H_2 -reception is associated with increased cAMP, H_1 -, with increased cGMP. Epinephrine also inhibits (50%) at $5 \cdot 10^{-5}$ M, independently of histamine. These two materials, then, result in decreased porcine epidermal mitosis, although activating adenylyl cyclase (267). The significance of cGMP as apparent effector in this porcine cell culture, as opposed to cAMP in human and mouse cell cultures, is unknown; it may be that different species have truly different signal systems with cAMP and cGMP in a given tissue, or possibly that measure of total cell cyclic nucleotides may not reflect the amount of receptor-bound nucleotide that is creating the observed effect (219). These are points of hot debate in the literature, currently.

Section 5

ANTIBIOTICS

5.1 Introduction

It has been suggested that an alternative to growth factors would be the incorporation of an antibiotic into an immediate burn covering. There are two essential reasons for this: 1) although no one really knows, it seems likely that a growth factor could operate more effectively in a post-excision covering than in an immediate one, and 2) that antibiotics have a remarkably greater preventive effect on bacterial colonization than a killing effect. In fact, from studies with iodophores releasing I₂, treatment of a burn within three hours is essential to kill skin flora; after this organisms are protected by the fibrinous coagulum which forms at the burn site (137). Later, the eschar formation (due to any unkilld flora) also serves to protect sub-eschar colonization from many topical antibiotics, and such growth is also somewhat resistant to systemic antibiotics. Hence, there is a double need for immediate biocide/biostat release from an immediate post-burn wipe-on.

Although silver sulfadiazine is the general topical agent of choice currently, there are several antibiotics available which might also be used. These have been reviewed recently (1981) (177,199,213), and therefore only the salient disadvantages to each are compiled here.

5.1.1 Antibiotic Evaluations: Silver Sulfadiazine (Silvadene)

This is only suppressive for gram positive bacteria. Rarely, it causes neutropenia.

5.1.2 Maleenide Acetate (Sulfamylon)

This causes pain upon application (245), skin rashes, and invariable systemic metabolic acidosis; also it is not very effective against fungi.

5.1.3 Gentamicin Sulfate (Garamycin)

This agent induces development of resistance; overabsorption may cause nephro- and oto-toxicity (281).

5.1.4 Nitrofurazone (Furacin)

This agent is not effective against gram negative bacteria. (However, its stellar performance against anaerobes makes it of interest if a covering would retard oxygen diffusion.)

5.1.5 Povidone-iodine (Betadine)

This agent is mildly to moderately painful upon application. It may induce sensitization.

5.1.6 Silver Salts: Nitrate, Allantoinate, Etc.

Silver salts must be frequently reapplied; they may cause severe electrolyte disturbance, and occasionally methemoglobin (by bacterial nitrate to nitrite conversion). In addition, silver nitrate solution stains. These characteristics are summarized in Table 5.1.

In addition to the above characteristics, diffusibility into the wound must be considered, both out of the covering and within tissue. Tissue diffusibility is high for Betadine, Furacin, and Sulfamylon, and lower for the other three drugs. Less is known about bulk diffusion within candidate covering materials; this field is reported in the next section.

Table 5.1

CHARACTERISTICS OF ANTIBACTERIAL CANDIDATES FOR AN IMMEDIATE POST-BURN COVERING

PROPERTY	SUBSTANCE					
	Silver Sulfadiazine (Silvadene)	Mafenide Acetate (Sulfamylon)	Gentamicin Sulfate (Garamycin)	Nitrofurazone (Furacin)	Providone-iodine (Betadine)	Silver Salts
Antibacterial Effects on gram () on yeast	- only* high	- only High on yeast; low on <u>C. albicans</u>	+, - High	+, - None	+, - Low	- Low
Stability	Yes	Yes	Yes, (may even be autoclaved)	Yes	Yes	Light unstable
Metabolic Disturbances	No	Acidosis	If over-absorbed only	No	No	Electrolyte imbalance
Diffusibility in Tissue	Low	High	Low	High	High	Low
Local Reactions	None	Yes	No	Rare	No	No
Eschar Separation	Normal	Prolonged	Normal	Normal	Normal	Prolonged
Concentration	1 %	10 %	0.1 %	0.2 %	≅ 3%	0.5 %
Pain on Application	No	Moderate	No	No	Mild-Moderate	Mild with nitrate, None with allantoinate

*One isolated clinician believes that Sulfamylon to be more effective against P. aeruginosa than Silvadene (282).

**Adapted from (213).

5.2 Incorporation Into Coverings

A number of the above materials have been incorporated into various covering materials, and their performance evaluated in vivo or in vitro. Iodine, the active ingredient in Betadine, has been comparatively tested in a wipe-on of PVP, poly(vinyl-pyrrolidone) (Povidone) vs. the nonionic surfactant Pluronic F-68 (137). Although both iodophores killed well, the F-68 appeared to protect bacteriocidal power longer than did the PVP. However, stability for three vs. five hours is hardly relevant to the present investigation, in which both an initial burst of activity and a subsequent lower level sustained release, over the intended life of the covering, might be considered useful. It was found that pH 2.1 solutions were much more bactericidal than pH 4.0 solutions; hence, a wipe-on of an iodophore of this form would require a rather low pH.

Another approach to iodine use, involved saturating a relatively thick (2 mm) silicone sheet with iodine (from alcohol solution). This study, as well as the one above, saw both early (initial kill) bactericidal advantage, as well as advantage appearing somewhat later, over control animals. The later effect, again, was probably due to prevention of eschar formation and consequent retained effectiveness of I₂ penetration (188).

Sulfamylon was incorporated in micronized form in a hydrophobic polymeric matrix, coated on a gauze. This vehicle underwent gradual biodegradation over 24 hours to release the drug at a smoother rate than by application of a cream (192).

Silver nitrate and silver sulfadiazine were soaked into collagen vs. amniotic membranes and evaluated for subsequent release. Uptake of ca. 1 mg silver per gram of collagen sufficed well to imbue bactericidal effects; silver nitrate was absorbed better by collagen, but silver sulfadiazine by the amniotic membrane (41). An earlier study had shown some incorporated activity from treating a nylon velour/polypeptide film laminated with silver sulfadiazine. This appeared to diminish gram-negative sepsis (however, the polypeptide film proved defective) (224). More recently,

silver sulfadiazine was cast at 10% into an acrylonitrile "copolymer" membrane, from which it leaked out in therapeutic amounts, and into a 2% "dry foam". The latter was more effective on supra-eschar guinea pig infections than was a 2 percent ointment (233).

In the same delivery style, an 8.5% mafenide dry foam exerted adequate suppression on P. aeruginosa, identical with that from an 8.5% ointment (232).

An early animal study (1973) dealt with replanted split thickness skin grafts, which reattached better if soaked in antibiotics (penicillin and streptomycin were used) for 15 minutes (173). This demonstrates that presence of other materials than the above six might also be considered (168).

Thus, it is evident that there is considerable likelihood of successful sustained release of several of these biocidal materials from an appropriate spray-, wipe-, or foam-on immediate post-burn covering.

Section 6

CONCLUSIONS AND RECOMMENDATIONS

Having surveyed the field of burn coverings, we draw the following conclusions as to the state-of-the-art.

First, post-excision burn coverings are at a high level of technology, and would appear presently adequate in quality. This has been achieved in products which allow partial or complete incorporability into the wound site, without the necessity of reclosure later with autograft. Further improvements, from tissue culturing of human skin, are likely, though not imminent.

Second, immediate post-burn coverings are at a technologically low level. Materials which exist have not been proved in practice, and the optimal parameters of immediate post-burn coverings have yet to be experimentally determined.

Third, the incorporation of growth factors and antibiotics into coverings, although fragmentarily researched for post-excision coverings, has been neglected altogether for immediate post-burn coverings. Trials of such multicomponent materials are needed; such materials clearly have the potential to confer benefits in the treatment of burn victims.

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Section 7

LITERATURE SEARCH TECHNICAL DATA

The following data bases were searched from 1977-present by the search terms listed.

MEDLINE Burns and any one of: collagen, fibroblasts, growth-substrates, insensible-water-loss, occlusive--or biological--dressings, polymers.

Also wound-healing and any one of: collagen, growth-substrates, occlusive--or biological--dressings.

NTIS Allograft.

Also burn and any one of: healing, toxin, treatment. Also skin and any one of: artificial, covering, (permeability or transmission) and (H₂ or water), regeneration.

Also (wound or burn) and any one of: covering, debridement, dressing, immune..., Macrophage or Lymphocyte or B-cell or T-cell, opson..., phytohemagglutinin.

Also Xenograft.

DTIC The following terms were requested, the actual terms searched were, however, not indicated on the printout: Allograft or Xenograft. Burn toxin. (Fibroblast or mesodermal or epidermal) and (growth-factors or EGF or FGF). T-(helper or suppressor) + cell or T-cell, B-cell, macrophage, mixed-lymphocyte-reponse or MLR, phytohemagglutinin-response or PHA, opsonic- α_2 -surface-binding-protein or SB. (Wound or burn) and any one of: (immune) and (system or response), covering, debridement, dressing, healing, treatment. (Artificial or synthetic) and (skin or covering). Water-vapor and (permeability or transmission) of human skin.

Science Citation Index

Searched 1977-April 1981 for the following: Burn (-s, -ed) and any one of: adherence, applied, approach, body-fluid, care, composite, covering, dressing(s), fabrics, fibroblast, following, formation, glutaraldehyde, healing, materials, mechanisms, new, opsonins, permeability, protecting, reconstruction, repair, skin, spray, synthetic, topical, toxin, treatment, water-loss, wound, wound-healing. Also skin and any one: allograft, analog, artificial, collagen, polymer, -grafts. Also wound and any one: adhesion, burn, covering, dressing, GAG, guanidine, infection, material, proline, tensile-strength, topical, treatment, -healing. Also angiogenesis and any one: factor, induce(-d), tumor. Also (first aid or immediate) and any one: burn(-s, -t), closure, protection, skin.

In addition to the above search, a special search for immediate post-burn coverings for 1970-1976 was made (in Science Citation Index) for the following: (burn or wound) and any one: application, covering, foam, gel, material, protect..., skin, spray, synthetic, topical, wipe-on.

Additionally to all of the above primary search, any references in the time period 1977 on cited by collected the collected references were also investigated.

The following generally relevant journals were also hand-checked from May 1981 on, through August 1981, if available:

American Journal of Surgery,
Journal of Biomedical Materials Research,
British Medical Journal
Journal of Investigative Dermatology,
Scandinavian Journal of Plastic and Reconstructive Surgery,
Journal of Plastic and Reconstructive Surgery,
Journal of Surgical Research,
Journal of Trauma, and
Annals of Surgery.

Section 8

SUMMARY OF INTERVIEWS HELD WITH RESEARCHERS ACTIVE IN DEVELOPMENT OF BURN WOUND COVERINGS

8.1 Introduction

Interviews were held with eight researchers active in development of materials for use as burn wound dressings. Identification of these workers was established by suggestions from Dr. C. Robert Valeri, Naval Blood Research Laboratory, Boston, and by perusal of current literature. These interviews were held for two purposes: 1) to present the Office of Naval Research with as current a picture as possible of the state of research and development in this area; and 2) to elicit from these workers their identification of research and/or development needs in burn therapy, especially with respect to dressings.

The workers with whom discussions were held are listed in Table 8.1 along with their institutional affiliations and their research interests relevant to burn dressings.

Table 8.1

INTERVIEWS

Dr. Andrew B. Walker	Amnion
Dr. Philip D. Thomson Shriners Burns Institute, Galveston	Amnion
Dr. Magdalena Eisinger Sloan-Kettering Cancer Research Institute, N.Y.	Epithelial Cell Culture
Dr. Howard Green Dept. of Biology, MIT	Rapid Epithelial Cell Culture
Dr. Eugene Bell Department of Biology, MIT	Living Skin Equivalent
Dr. John Burke Shriners Burns Institute, Boston	Artificial Skin

Table 8.1 (Continued)

Dr. Charles R. Baxter
University of Texas Health
Science Center, Dallas

Dermal
Transplants

Dr. Basil A. Pruitt
Brooke Army Medical Center
Fort Sam Houston, Texas

Metabolic
Responses to
Injury

Three major areas of current research activity were identified:

1. Development and Use of Amnion as a Temporary Biological Dressing.

Researchers interviewed were Drs. Andrew B. Walker (Allentown Hospital, Allentown, PA) and Philip K. Thomson (Shriners Burns Institute, Galveston, Texas).

2. Development and Clinical Trials of an Artificial Skin. This precipitated collagen film backed with silicone, developed by Drs. John K. Burke (Shriners Burns Institute, Boston, Massachusetts) and I. V. Yannas (Department of Mechanical Engineering, MIT) is currently under clinical trial. This material takes the place of autografts.

3. Epidermal Tissue Culture in Conjunction with Dermal Substitutes.

Workers interviewed have taken different approaches to developing methods for use of materials which combine absorbable dermal substitutes with overlaying cultured epidermal layers.

- Dr. Charles R. Baxter (University of Texas Health Science Center, Dallas, Texas) uses non-viable dermal layers harvested from cadavers as an absorbable biological dressing overlaid with cultured epidermis.
- Dr. Howard Green (Department of Biology, MIT) has developed techniques for rapid epithelial cell culture.
- Dr. Eugene Bell (Department of Biology, MIT) has developed methods for seeding collagen films with fibroblasts, a technique which encourages restructuring of the collagen as new collagen replaces the biological dressing. Although the approach taken by those workers differ, the common goal is to obviate the need for autografting.

Discussion with Dr. Basil Pruitt (Brooke Army Medical Center, Fort Sam Houston, Texas) provided insights into problems encountered in treatment of injuries especially the institutional structures available for dealing with trauma. Dr. Pruitt's research interests do not directly center on burn coverings.

Dr. Magdalena Eisinger (Sloan-Kettering Cancer Research Center, New York City) agreed to be interviewed but during the discussion it became apparent that she was unwilling to discuss her current research.

The information garnered from each interview is summarized in Table 8.2. This table consists of eight pages, each devoted to one worker.

Section 8.2 elaborates on some significant aspects of work concerned with burn coverings designed to be bioabsorbable. This includes development of collagen based materials, or dermal sheets which can be used in conjunction with cultured epidermal layers. In addition to discussion of current research, part of each interview was devoted to discussing areas in burn therapy which those workers identified as requiring further research and development efforts.

Section 8.3 summarizes interviews with workers concerned with amnion.

Section 8.4 includes a statement of research and development needs as identified through those interviews. This section also includes recommendations which are solely derived by the Dynatech personnel responsible for this report as a synthesis made from these interviews.

Table 8.2

Summary of Interviews Held with

Dr. John Burke
Dr. Eugene Bell
Dr. Howard Green
Dr. Andrew B. Walker
Dr. Philip K. Thomson
Dr. Charles R. Baxter
Dr. Basil A. Pruitt
Dr. Magdalena Eisinger

DR. JOHN BURKE

Shriners Burns Institute, Boston

Artificial Skin

- Cross-linked collagen-glycosaminoglycan co-precipitate, backed with silastic film.
- Storable dry or in 70 percent IPA, at room temperature.
- Fits contours of wound.
- Is permanent replacement for excised skin; no rejection; absorbed in 2 months.
- Reduces scarring but does not eliminate it.
- This material possibly used as immediate dressing; has analgesic properties but preference is for no treatment.

Current Status of Research

- Material in clinical trial; published in Ann. of Surg., 1981 (282).

Further Developments

- Possibly desirable to incorporate additives such as antibiotics, hormones, antibodies, growth factors into the artificial skin.

Table 8.2 (Continued)

DR. EUGENE BELL

Department of Biology, MIT

Research Relevant to Wound Therapy

- Preparation of Collagen Lattices seeded with fibroblasts.
- Epidermal layer seeded onto collagen from disaggregated cell culture. These grow to confluence and form multi-layered epidermis.
- Graft dessication minimal due to keratinization of upper cell layers.
- Graft not sutured; held in place by wet bandage during "take"; no visible seam between graft and original tissue.

Recent Research Findings (to be published)

- Mitotic activity of fibroblasts in lattice stops in vitro but cellular metabolism does not. In vivo mitotic activity continues.
- Synthesis of cellular products governs remodeling of collagen dermal layer as evidenced by birefringence studies.
- Rapid method of generating a dermal equivalent (dermal layer) has been developed and depends upon the use of blood platelets as substitutes for dermal cells. Up to 0.5 m² can be prepared within six hours of receipt of 1 unit of blood.

Fruitful Avenues of Research and Development

- More rapid proliferation of epidermal layers.
- More rapid restructuring of collagen by dermal components.

Table 8.2 (Continued)

DR. HOWARD GREEN

Department of Biology, MIT

Accomplishments

- Rapid culture of epithelial cells from disaggregated cell suspensions using EGF.
- Sufficient confluent multi-layered epidermal sheets can be prepared in 3-5 weeks for grafting high percent TBSA wounds.
- Sheets contain viable cells in all stages of differentiation.
- Work now in clinical trials.

Research Plans

- Typing of cell cultures for use of allografts. Epidermal sheets could be stored frozen; with 50 percent viability on thawing, sufficient for grafting.

Fruitful Avenues of Research and Development

- Development of polymeric barrier films for controlling water loss and infection prior to hospitalization.

Table 8.2 (Continued)

DR. ANDREW B. WALKER

Allentown, PA

Accomplishments

- Development of new methods of collection, processing, and storage.
- Demonstration of analgesic effect of amnion on second-degree burns.
- Amnion used as covering for partially excised second-degree burns, as temporary dressing on third-degree burns, on skin ulcers.
- Establishment of amnion bank.

Clinical Observation

- Amnion sometimes obviates need for grafting deep second-degree burns.

Present Status of Research

- Preliminary experiments for long term preservation by chemical treatment.

Table 8.2 (Continued)

PHILIP D. THOMSON

Shriners Burns Institute, Galveston

Research Relevant to Burn Therapy

- Monitoring, banking, and clinical use of amnion as a burn wound dressing.
- Use of amnion in conjunction with meshed autografts.
- Amnion suitable as immediate dressing on superficial second-degree burns.
- Temporary covering on recently excised wounds prior to autografting.

Other Research Interests

- Surgical management of burn wound sepsis.
- Immunological responses of body, after severe injury.
- Problems on use of porcine xenograft.

Table 8.2 (Continued)

DR. CHARLES R. BAXTER

University of Texas Health Science Center

Recent Research Interests

- Study of Povidone-Iodine absorption into burn wounds.
- Dependence of burn materials on Leukocyte function.
- Abnormal erythrocyte morphology in burns.
- Studies on fluid resuscitation.

Research Relevant to Wound Therapy

- Use of non-viable dermal layers harvested from cadavers as permanent dermal graft.
- Dermal layer covered during vascularization with plastic shield (Op-Site).
- Replacement of plastic shield with cultured epidermal layer via tissue cultures.

Fruitful Avenues for Research and Development

- Polymer platen for epidermal cells.
- Methods of chemically treating harvested skin.
- Immediate first aid material with SR.

Table 8.2 (Continued)

DR. BASIL A. PRUITT

Brooke Army Medical Center
U.S. Army Institute of Surgical Research
Fort Sam Houston, Texas

Research Interests and Findings

- Metabolic response to injury; hyperthermia not a response to evaporation loss. Metabolic rate in burn victims correlated with catecholamine, cortisol, and glucagon secretion (not yet published).
- Studies in area of host resistance including bacterial motility.
- Effectiveness of antibiotics as a function of their physical properties.
- Development of topical chemotherapeutic agents used to control infections in burn wounds.
- Evaluation of allografts and xenografts as biologic wound dressings.
- Studies of bilaminate material developed by Dr. Norman Levine.

Suggested Research and Development

- Identification of pharmacological agents which might decrease permeability of injured capillaries and thereby keep fluid within circulation.
- Protective first aid coverings with analgesic properties, and broad spectrum antibiotic to prevent formation of a closed infected wound.

Table 8.2 (Continued)

DR. MAGDALENA EISINGER

Sloan-Kettering Institute for Cancer Research

Accomplishments

- Growth of multi-layered epidermal sheets from single cell suspensions.
- Claims that epidermal growth and differentiation occurs in absence of dermal components or special nutrients.
- Uses collagen film support.

Present Status of Research

- Will not discuss

8.2 Interview Summaries: Research on Absorbable Coverings Designed to Obviate the Need for Autografting

Although the approaches described in this section differ, they have in common the goal of surgical repair of burn injuries which eliminate the need for auto grafting. Work in this area includes development of bioabsorbable materials (collagen, collagen-based, or harvested dermal tissue). In some cases these were intended to be used in conjunction with cultured epidermal layers. Work described in this section also includes emphasis solely on epidermal tissue culture.

Interviews, the summaries of which are included in this section, were held with Drs. Baxter, Bell, Green, and Burke. The summary of our interview with Dr. Pruitt is also included here.

Dr. Baxter. Dr. Baxter has clinically demonstrated that non-viable dermal layers harvested from cadavers may be used in place of autografted skin as a permanent dermal graft. These dermal layers are subject to several freeze-thaw cycles so that the viable cell content is virtually nil. During vascularization of the dermal layer, moisture loss is controlled by Op-site. When the dermal graft has taken, the Op-site shield is replaced by a confluent epidermal layer prepared from the patient's own tissue.

Dr. Baxter has identified several research priorities. Two of these stem from his clinical experience.

Cultured epidermal sheets would be more conveniently grown on a polymeric plate which would serve as both a support for the cells during growth and as a moisture and bacteria controlling barrier after the cell layer has been placed in contact with the dermal underlayer. The platen would have to be flexible and inert, its surface characteristics designed to stimulate or support epithelial attachment and growth, and its permeability adjusted to govern passage of moisture. Such platens do not at the present exist.

The second need identified is for methods of chemical treatment of harvested skin (dermal layers) for long-term storage at room temperature.

Dr. Baxter also realizes a need for a superior first aid treatment. A polymeric barrier for control of water loss and bacterial infection would be desirable for immediate post burn treatment. Control against infection could be achieved by sustained release from the polymer of suitably chosen agents.

Dr. Bell. Dr. Bell's living skin equivalent is a collagen bed seeded with dermal fibroblasts which continue to function biosynthetically and add structural components to the original collagen lattice. Dr. Bell has recently developed a method for rapidly generating a dermal equivalent seeded collagen bed; up to 0.5 m² with the use of blood platelets instead of cells. This can be prepared within six hours of receipt of one unit of blood. Thus, Dr. Bell's preferred directions of research and development include:

- techniques for more rapid proliferation of the epidermal layer; and
- induction of more rapid restructuring of the collagen by the dermal components.

Dr. Green. Dr. Green has developed methods for rapidly culturing confluent multilayered epidermal sheets with viable cells in all stages of differentiation. Although culture of epidermal cells is employed widely, Dr. Green's important contribution is the rapidity with which such confluent layers may be generated. His methodology includes use of epidermal growth factor and can result in preparation of sufficient material within three to five weeks for grafting high percent total body surface area burns. These sheets are now in clinical trial.

His priorities for research and development include typing of cell cultures for use of allografts. Typed epidermal sheets could be grown,

and stored frozen for eventual use; cell viability on thawing would be approximately 50 percent.

A second area deserving research and development attention is development of a polymeric barrier for protection of burned tissues prior to hospitalization. The function of the first aid method would be protection of the traumatized area against infection and control of water loss from the burned area.

Dr. Burke. The artificial skin developed over the past ten years by Drs. Burke (Massachusetts General Hospital, Boston, MA) and I.V. Yannas (Dept. of Mechanical Engineering, MIT) is a unique contribution to the field of burn covering research. The Burke/Yannas material is collagen coprecipitated with glycosaminoglycan. The goal of this work now realized, is a bioabsorbable material engineered to have the flexibility needed to conform easily to a wound bed. Water permeability is controlled by a thin Silastic backing which sloughs off as the material is absorbed. Healing is accompanied by incorporation of or absorption of the synthetic collagen into new tissue.

The Burke/Yannas synthetic skin overcomes storage problems. It has an indefinite shelf life either dry or when kept in 70 percent isopropanol. Storage in isopropanol keeps the material sterile and flexible for immediate use; dry storage entails several minutes hydration before use.

Dr. Burke has observed the analgesic effect of the artificial skin when applied directly to burns of less than 3rd degree severity.

Although Marion Laboratories has been licensed to manufacture the material, supplies are limited and apparently will be so for some time.

Dr. Burke's artificial skin is presently in clinical trial. Although the present "second generation" material, incorporating seeded basal epidermal cells, appears to yield clinically satisfactory results (see

section 3.5), Dr. Burke may in the future investigate the effects on healing of materials incorporated into the skin. Additives might include certain hormones, antibodies, or growth factors.

Dr. Pruitt. Dr. Pruitt was involved in the development of the original topical chemotherapeutic agent used to control infection in burn wounds and has also been involved in the clinical evaluation of allograft and xenograft skin used as biologic wound dressings. He has collaborated in studies of a bilaminate material developed by Dr. Norman Levine of the U.S. Army Institute of Surgical Research.

His opinion on preferred directions for R/D effort include:

- Development of pharmaceutical agents which would decrease the permeability of injured capillaries. Such agents would reduce the rate of pervaporative water loss from burned tissues; and
- Dr. Pruitt has also identified a need for a protective first aid covering. A polymeric film including both an analgesic agent and a broad spectrum antibiotic is a desirable immediate first aid treatment.

8.3 Interview Summaries: Amnion (Amniotic Membrane)

Two of the workers interviewed, Drs. Walker and Thomson, were interested in exploiting amniotic membrane as temporary biological dressing prior to autografting. The reasons for this interest appear to be concerned with the following factors:

- Supply. Human amnion is at present available through hospital maternity units. It may be stored refrigerated or frozen until needed. Its sterility may be maintained by storage in penicillin solution. Presently, these methods are sufficient but supply is limited and demand for the material is at least as great as supply. Thus, long-term storage methods have not

been necessary. Supply, however, may be greatly increased by making use of bovine amnion which may be harvested in conjunction with preparation of fetal calf serum. Although we have not investigated the extent of this potential supply we are assured that it is considerable.

- Physical Properties. The mechanical properties (water permeability, flexibility, and barrier to bacterial invasion) make amnion suitable as a temporary dressing prior to autografting, or in conjunction with autografting.

Workers have noted that amnion, when placed on burns of less than third degree severity display an analgesic effect. Although the intent of the current work is not directed toward investigating this property, it points to the potential utility of amnion as an immediate post-burn (first aid) treatment.

Dr. Walker. Dr. Walker has used amnion as temporary dressings for prepared wound beds prior to autografting. He has observed that amnion sometimes obviates the need for grafting deep 2nd degree burns which suggests that amnion may prevent progressive damage to tissues. In addition, Dr. Walker has used amnion as dressings for partially excised 2nd degree burns, and as temporary dressings, 3rd degree burns and skin ulcers.

Dr. Walker has established an amnion bank at Allentown and in conjunction with this activity has developed methods for collection, processing, and storage of amnion.

His research priorities include development of methods for amnion preservation by chemical treatment.

Dr. Walker has observed the analgesic properties of amnion.

Dr. Thomson. Dr. Thomson is concerned with problems of monitoring sterility of amnion, in storage or banking of amnion as well as in

its clinical use in conjunction with surgical repair of burn injuries. He also has commented on the suitability of amnion as an immediate dressing on superficial 2nd degree burns.

8.4 Conclusions and Recommendations

In this section are listed the areas identified directly or suggested by the interviewees as needing further research and development. Included also is a synthesis made by the interviewer, Dr. Gresser, Dynatech R/D Co. The recommendation embodied in this synthesis is solely the responsibility of those who prepared this report.

Our understanding of the work of Burke, Bell, and Green suggests that each of these investigators has contributed importantly to the development of absorbable burn coverings. Each has brought his work to the point of clinical trial; however, if synthesis of aspects of the work could be made by a cooperative enterprise, it might be possible to bring about even greater advance.

Dr. Bell has developed a method for rapidly generating collagen beds seeded with fibroblasts. Although we have no evidence to support this speculation, it may be that Dr. Bell's collagen bed does not have the flexibility and "drapability" of Dr. Burke's collagen coprecipitated with glycosaminoglycan. Dr. Burke's synthetic skin has been engineered so that conformity to the wound bed is improved. The obvious area of collaboration is to improve preparation of the collagen beds by combining the rapid production and seeding techniques employed by Bell with the characteristics engineered into Dr. Burke's collagen.

Dr. Burke did not speak of using epidermal sheets in conjunction with his membrane, although this is an integral aspect of Dr. Bell's living skin equivalent. Dr. Green's interests did not include development of collagen beds to receive the multilayered epidermal sheets which he has been able to produce more rapidly than allowed by conventional techniques. The application of Dr. Green's techniques . . . rapid epidermal culture in

conjunction with the improved product of Dr. Burke's and Bell's collaboration methods would in our opinion give rise to an absorbable burn covering superior to those discussed above.

As listed in Table 8.2, Dr. Bell has suggested that techniques for more rapid proliferation of the epidermal layer are desirable as is development of means for inducing more rapid restructuring of the collagen by dermal components.

8.4.1 Suggestions Made by Interviewees for Research and Development

The following recommendations for fruitful avenues of investigation have been abstracted from the interviews. The originators of each suggestion are identified in the text.

- Development of Polymeric Barrier Films as a First Aid Treatment for Burn Victims. The aim of this treatment would be to control water loss and to minimize bacterial invasion. (Green, Baxter, Pruitt). Desirable attributes would be analgesic properties to minimize pain and incorporation of sustained release agents such as broad spectrum antibiotics to prevent infection (Pruitt). Dr. Burke also has considered addition of specific agents to his artificial skin.
- Development of a Polymeric Platen for Support of Cultured Epithelial Sheets (Baxter). Culture and use of epithelial sheets for deposition on dermal layers; i.e., collagen or harvested dermal layer would be facilitated by a polymeric platen with surface characteristics to encourage epithelial proliferation and to support confluent epidermal sheets. This platen would have to have a controlled permeability to water in order to allow adequate transpiration from the attached cell layer without dessication.

- Chemical Methods of Tissue Preservation. The use of amniotic membrane as a biological dressing and of absorbable dermal layers harvested from cadavers may become viable treatment modes. The potential availability of both tissues seems to be abundant. Chemical treatment of amnion for preservation and storage at room temperature would be desirable (Walker). Methods for collection, preservation and storage of amnion should be developed (Thomson).

Dermal tissue harvested from cadaver skin is used by Baxter as a bioabsorbable covering for full thickness excisions. Dr. Baxter suggests that methods of chemical treatment for preservation should be developed.

- Cell Physiology. Several studies in cell physiology were suggested. These include developing methods for typing cell cultures so that cultured epidermal sheets for grafting would be immediately available in banks (Green).
- Developing methods for inducing more rapid restructuring of collagen layer by seeded dermal components (Bell).

8.4.2 Recommendations for Research and Development

Several workers have commented on the analgesic properties of these burn coverings when applied to burned tissues prior to debridement; that is, when applied immediately after injury and before surgical intervention. Dr. Burke has observed this effect with his artificial collagen based skin, Drs. Walker and Thomson have also commented on this with respect to amnion.

It is clear that this property could be used to great advantage in the immediate post burn treatment of burn victims. First aid coverings based on collagen or amnion would have functions other than pain reduction:

including control of water loss and prevention of infection. These functions might in part be met by allowing the membrane to serve as a reservoir for the sustained release of active agents including but not necessarily limited to antibiotics.

Successful exploitation of these materials would require the following effects.

- Survey the potential supply of amnion from human and bovine sources. The former would be available through hospitals, the latter would be collected as an adjunct to harvesting fetal calf serum.
- Develop methods for chemically treating amnion for long-term storage at room temperature. Such treatment obviously must not interfere with the analgesic properties.
- Since Dr. Burke's precipitated collagen and amnion both display analgesic properties, it is probable that cadaver skin, or the dermal layers harvested there from also do. Chemical preservation of this tissue should be explored conjointly with methods for amnion. (See Dr. Baxter's suggestion.)
- Develop methods for employing collagen films, cadaver skin, and amnion as reservoirs for sustained release of chosen agents.
- Develop methods for controlling permeability of these treated materials for control of moisture transmission rate.
- Explore applicability of Dr. Bell's method for rapid generation of collagen films to production of quantities sufficient for use in first aid.

Section 9

RAT TESTING

9.1 Objectives

The purpose of this series of rat tests was to examine quantitatively the adherence of burn coverings to the fully excised backs of rats. The data generated allow quantitative comparisons to be made of a variety of coverings in use today.

One problem with the present program was that not all coverings were available to us. For example, the collagen skin developed by Burke and Yannas was in critically short supply so it could not be tested. The second problem was that not all coverings were adaptable to this type of protocol. Plurionics-F127, a gel covering, was unsuitable for removal by peeling.

9.2 Experimental Methods

Four coverings were evaluated in this test: Biobrane (Hall-Woodroof), Second Skin (Spenco) (used with and without the film removed from the exposed side), and Plurionics-F127 (BASF Wyandotte). Animal surgery and husbandry was carried out at SISA, Inc., Cambridge, Massachusetts, under the direction of Dr. John Howes.

On day 0 male and female CD rats (from Charles River Rat) weighing 240-260 grams were anesthetized with Nembutal I.P. (50 mg/kg). A 5 x 7.5 cm² to 5 x 8.5 cm² area of skin was removed from the rat's back with surgical scissors. The hair was not removed prior to excision. A piece of wound covering was cut to fit the excised area, placed on the area, and secured with ~12 wound clips. Four rats were used for each covering. The animals were returned to their individual cages and allowed food and water ad libitum for four days. The only exception was the four Plurionics-F127 rats (20 g Plurionics, 80 g distilled H₂O, stirred on ice for 4 hours prior to application, Schmolka 1972). After excision, the gel was applied to the

exposed area, and the rats returned to their cages. After 24 hours the four Plurionics-F127 rats were not eating as well as the other 12 rats and appeared to be in pain. These rats were sacrificed with an euthanasic dose (100 mg/kg) of Nembutal. The gel covering would not have been adaptable to Instron testing anyway, so these sacrifices were not considered detrimental to the experiment.

On day four the rats were brought to Dynatech R/D Company for adherence testing on the Instron Tensile Tester (Instron TT-C Universal Tester Model, Instron Corporation, Canton, Massachusetts).

The rats were given 50 mg/kg Nembutal I.P. prior to the test. In preparation for the testing, the wound clips were removed and the edges of the covering lifted. The edges were trimmed to remove the encrustation that had formed around the edges so that the covering was approximately 4.5 cm wide.

Rats were then mounted on the rat board shown in Plate 9.1. The head of the rat was pushed through the 1" nylon tubular webbing harness and a piece of duct tape was tightly attached to both the rat and the harness to prevent slippage. A second piece of duct tape was used to secure the posterior end of the rat. This mounting is shown in Plate 9.2, which shows a covering being removed from the rat's back during a test. Next, the rat arm, shown in Plate 9.3, was positioned so that the clip was even with the posterior end of the covering. The covering was then attached to the clip. The Instron Tester was started and the covering was slowly removed as the test proceeded. Data was recorded on a strip chart and saved for later analysis along with pertinent observations. Many of the rats died during the test, but all were given a second dose of 50 mg/kg Nembutal I.P. for euthanasia.



Plate 9-1

Rat Board



Plate 9-2
Rat Test in Progress, Showing Entire Assembly

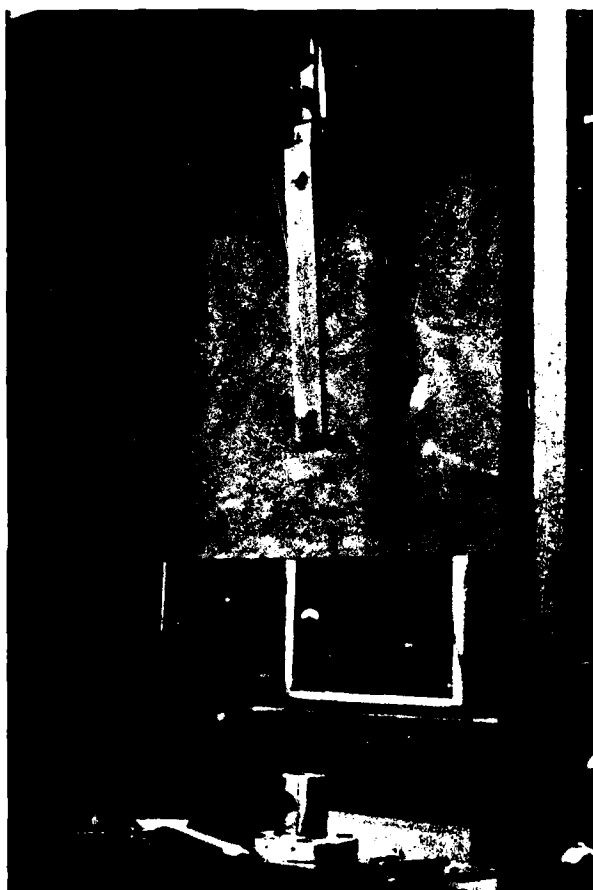


Plate 9-3
Rat Arm

9.3 Analysis of Instron Data

The raw data generated by the Instron Tensile Tester must be properly interpreted in order to yield useful parameters such as adherence. This section describes the calculations involved in that exercise.

The data from Instron Tensile Tester is recorded on a strip chart ten inches wide and marked in inches lengthwise. First, a representative section of the "curve" must be chosen. That is, a section where the width of the bandage was consistent, where the edge of the clip did not catch in the covering, or where the test was not just beginning. The baseline of the print out must be checked to make sure that it was properly zeroed; if it was not, as in Figure 9.1, the baseline must be drawn in. After the ends of the representative section of the curve are marked and the baseline corrected, the area under the curve is measured with a planimeter. The length of the area is also noted. Three other parameters, jaw separation rate (in/min), chart speed (in/min), and scale (lbs/in), also need to be known. For example a scale of 2.0 lbs/in means that each inch of the ten inch wide chart is equal to 0.2 lbs.

With these facts, adherence may be calculated:

$$\text{Adherence (lbs/in)} = \frac{A \cdot S \cdot F}{L \cdot W \cdot F}$$

where: A = area under the curve (in²);
S = scale (lbs/in);
F = chart speed divided by jaw separation rate
(units cancel out);
L = length of the area (in); and
W = wound covering width (in).

9.4 Results

The actual chart recordings of the tests are presented in Figures 9.1 - 9.12. This data was interpreted as discussed in Section 9.3,

Figure 9.1

Biobrane Adherence Test
(Test No. 1 on Table 9.1)

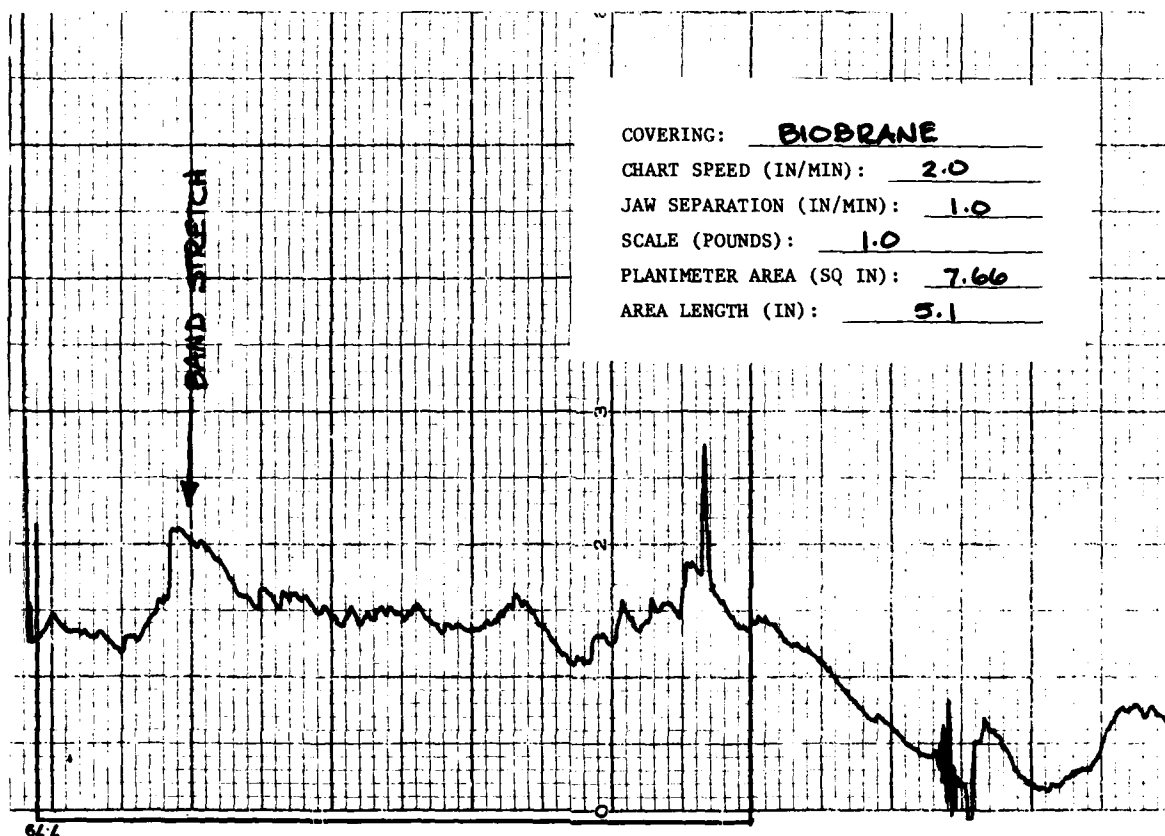


Figure 9.2

Biobrane Adherence Test
(Test No. 2 on Table 9.1)

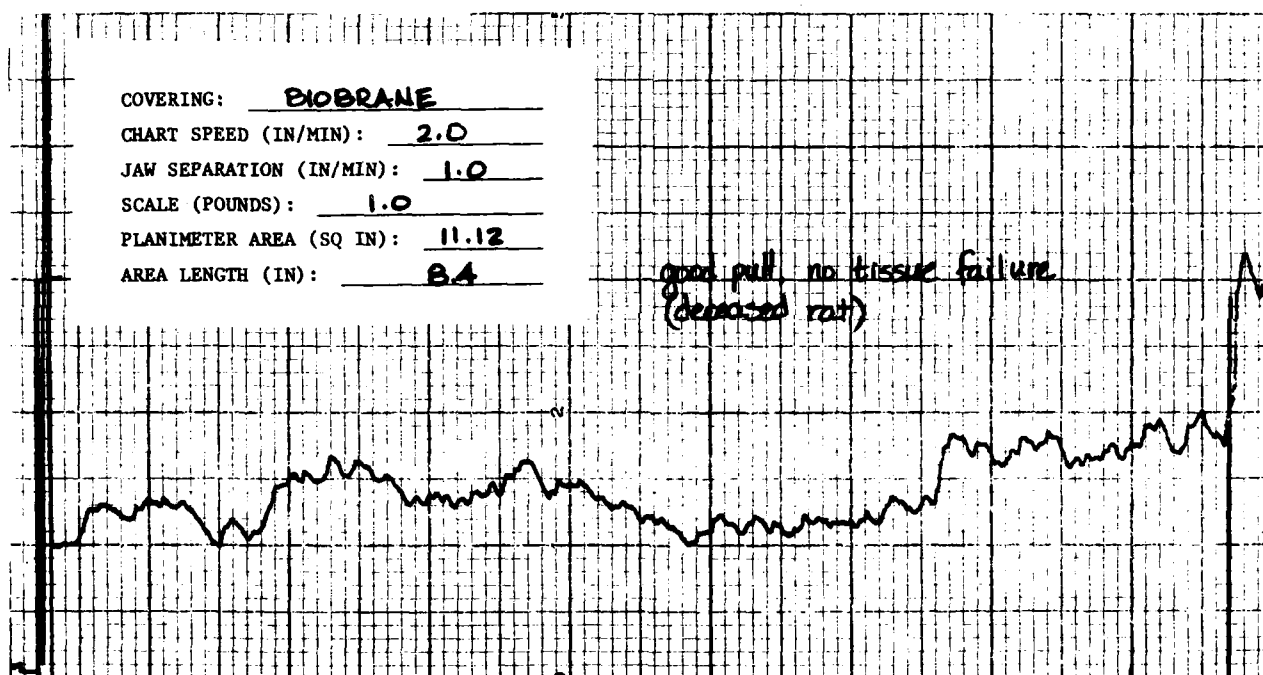


Figure 9.3

Biobrane Adherence Test
(Test No. 3 on Table 9.1)

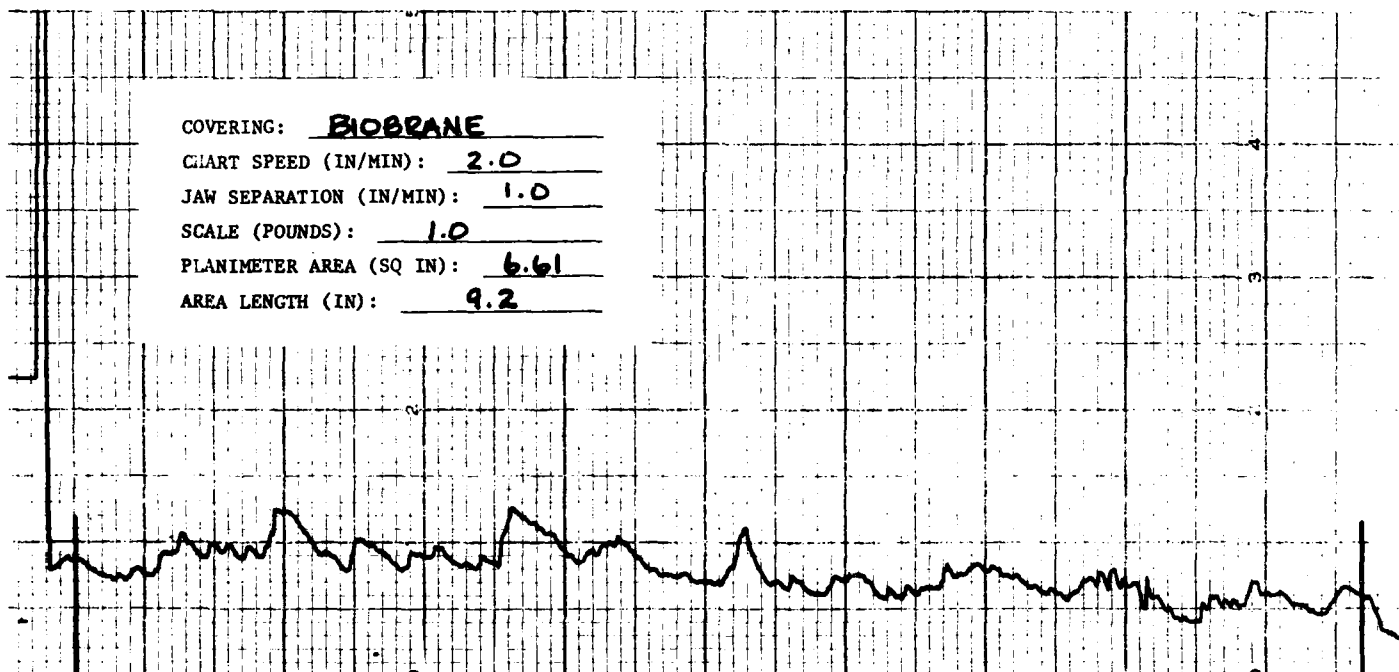


Figure 9. 4

Inverted Biobrane Adherence Test
(Test No. 4 on Table 9.1)

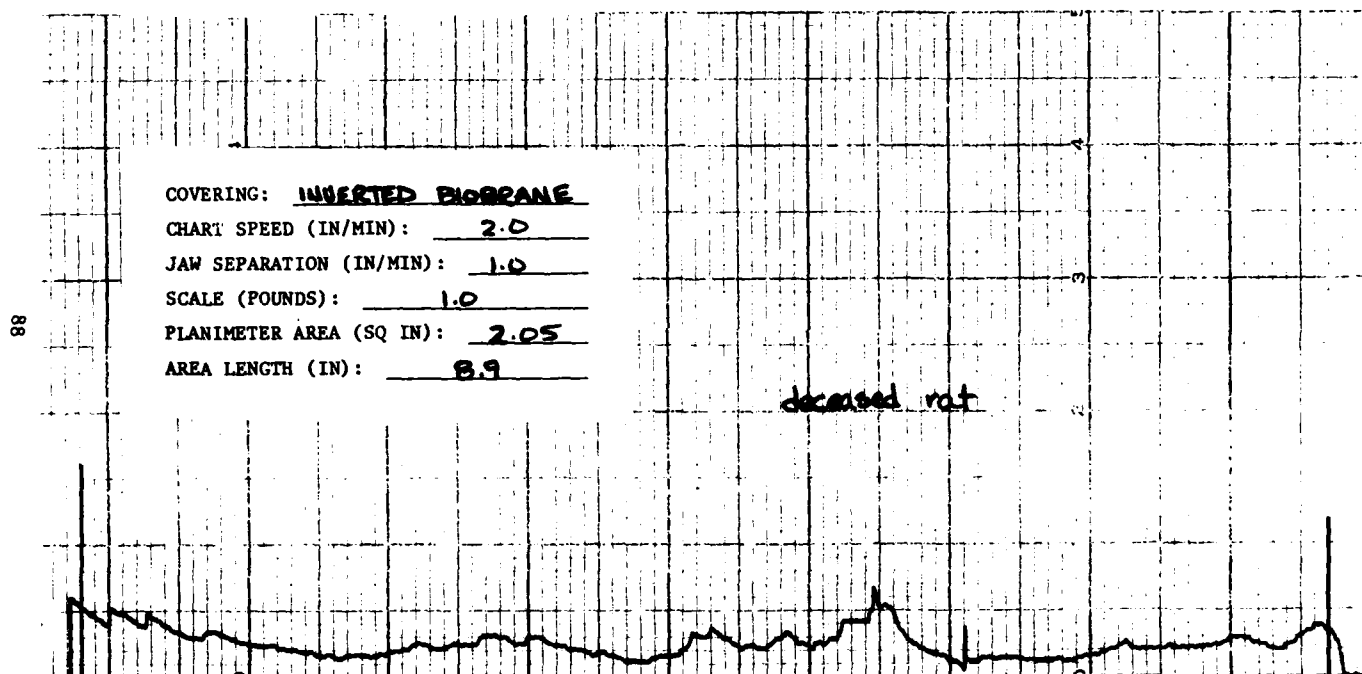


Figure 9.5

Second Skin (Film On) Adherence Test
(Test No. 5 on Table 9.1)

68

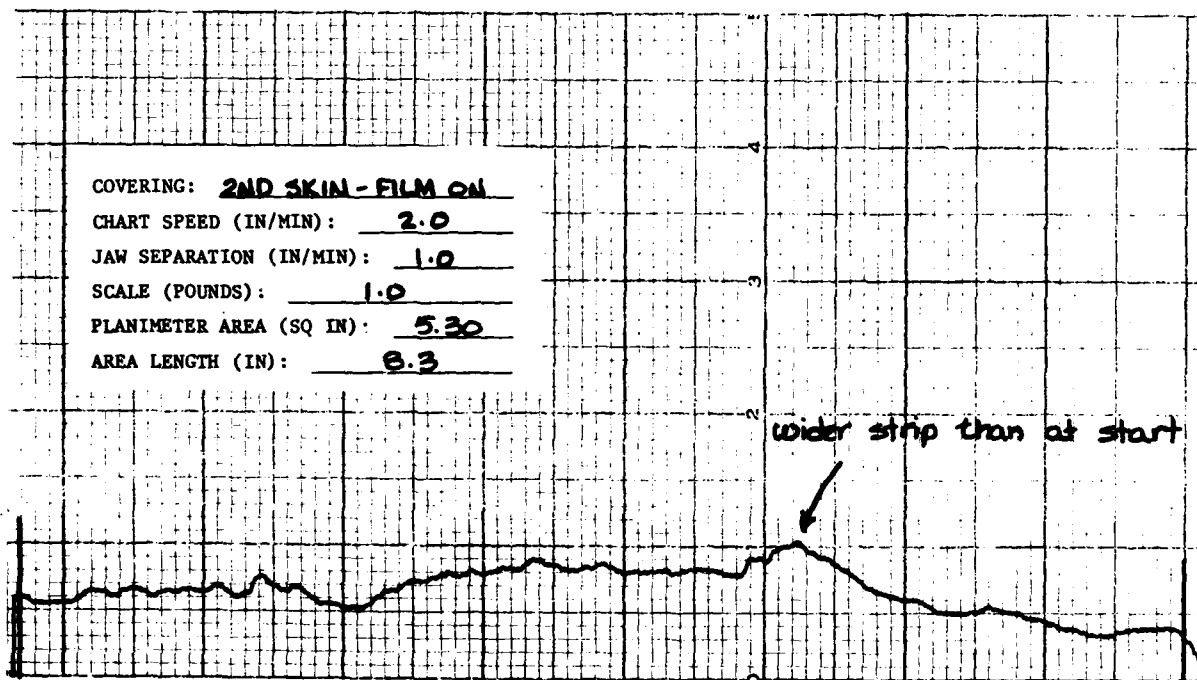


Figure 9. 6

Second Skin (Film On) Adherence Test
(Test No. 6 on Table 9.1)

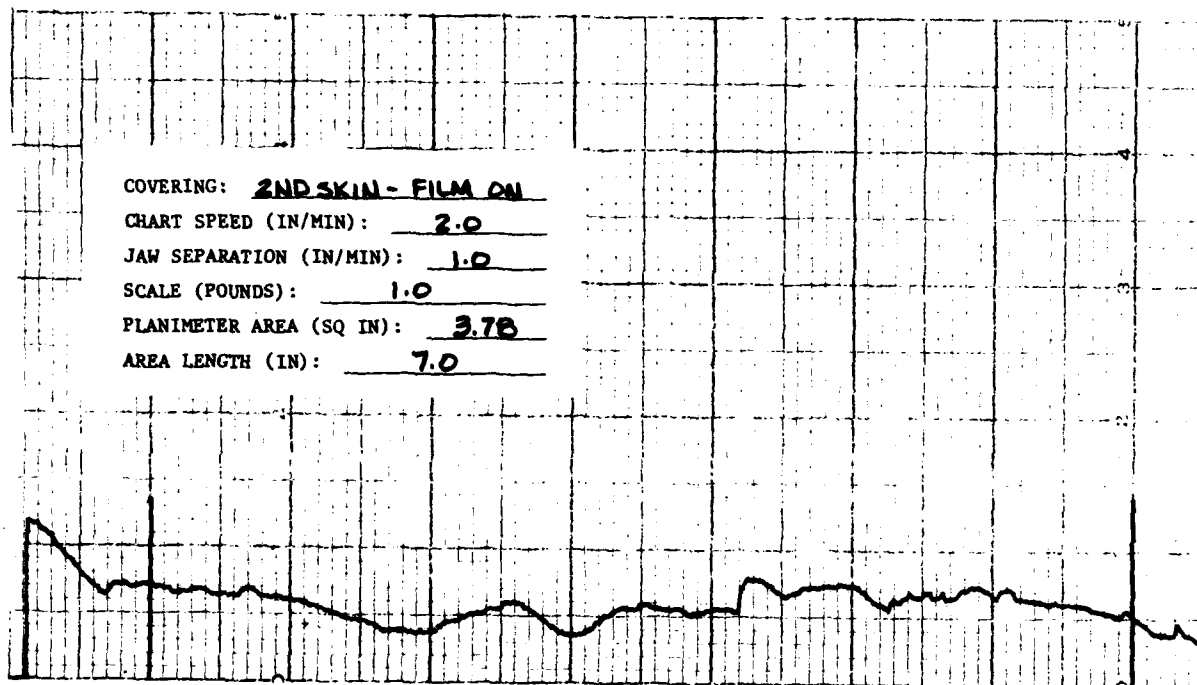


Figure 9.7

Second Skin (Film On) Adherence Test
(Test No. 7 on Table 9.1)

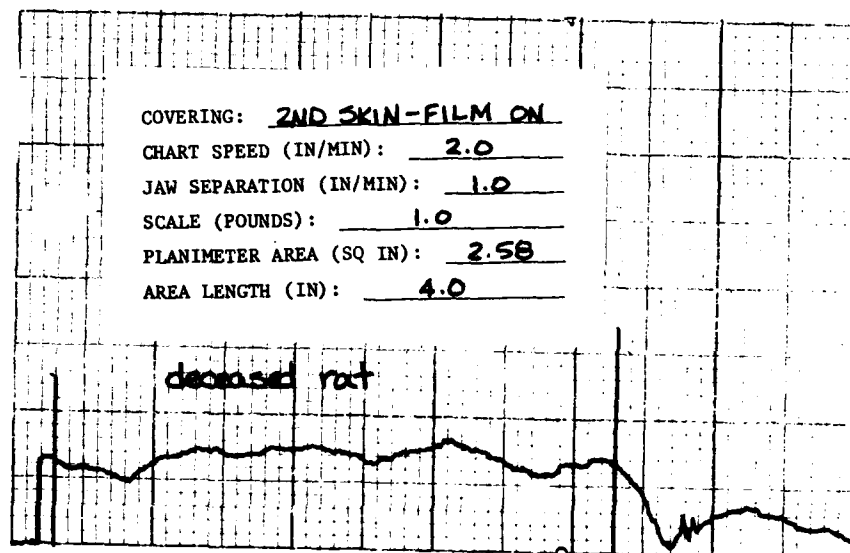
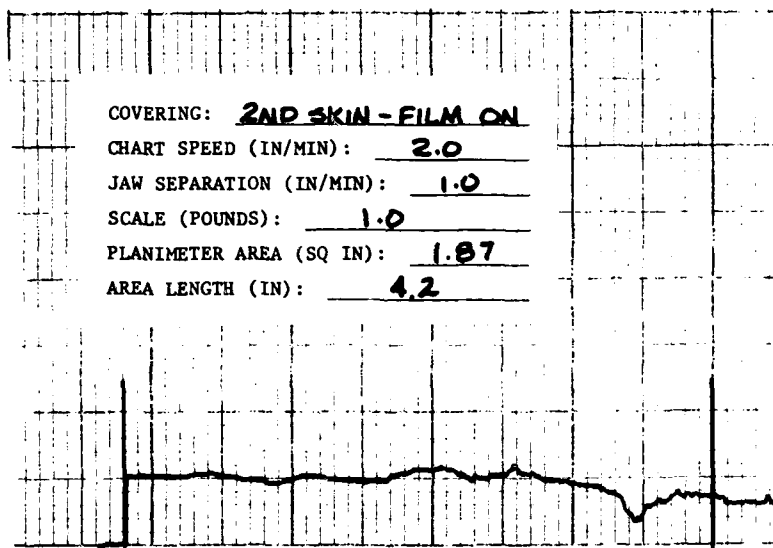


Figure 9. 8

Second Skin (Film On) Adherence Test
(Test No. 8 on Table 9.1)



AD-A117 879

DYNATECH R/D CO CAMBRIDGE MASS
ASSESSMENT OF WOUND THERAPY SYSTEMS.(U)
MAY 82 S DE RIEL, J D GRESSER, C E WEST
DYNATECH-2165

F/G 6/5

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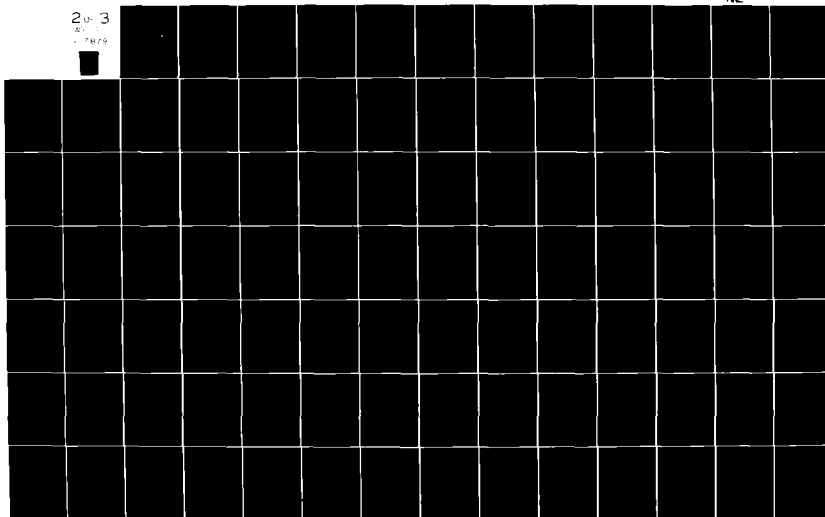


Figure 9.9

Second Skin (Film Off) Adherence Test
(Test No. 9 on Table 9.1)

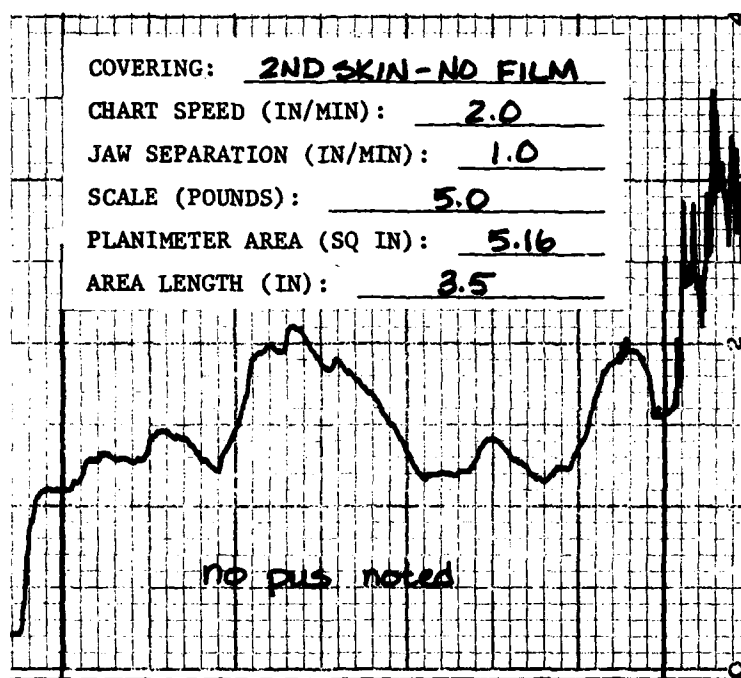


Figure 9.10

Second Skin (Film Off) Adherence Test
(Test No. 10 on Table 9.1)

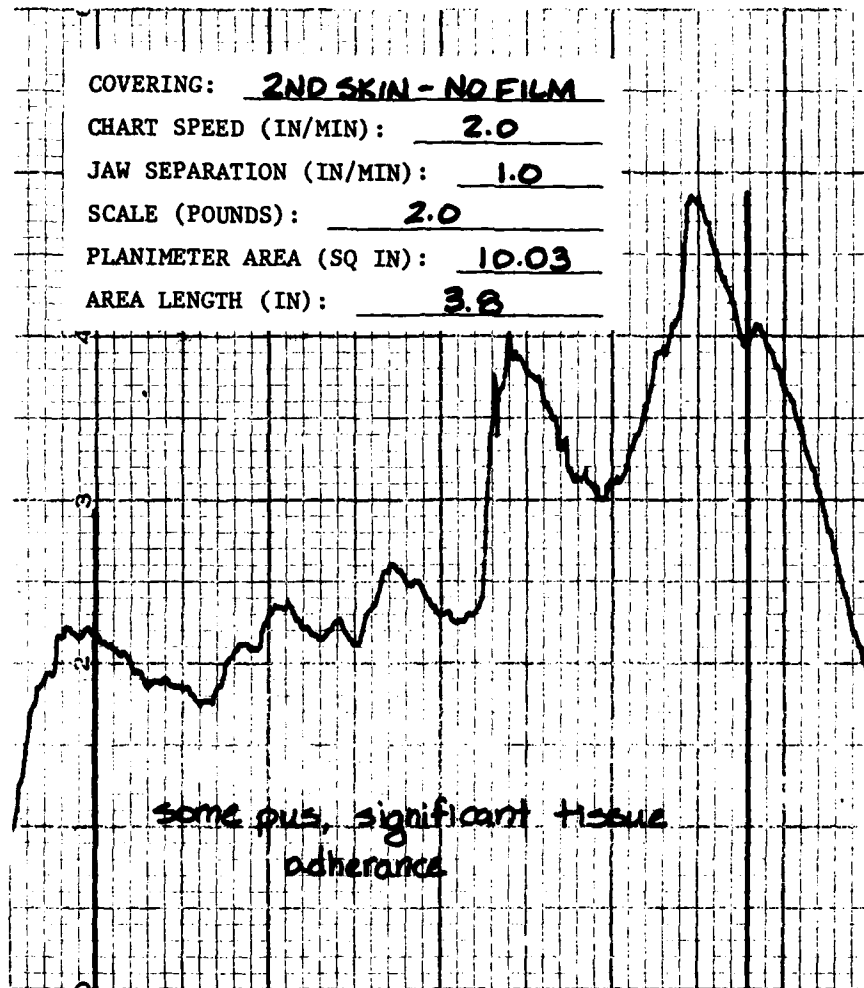


Figure 9.11

Second Skin (Film Off) Adherence Test
(Test No. 11 on Table 9.1)

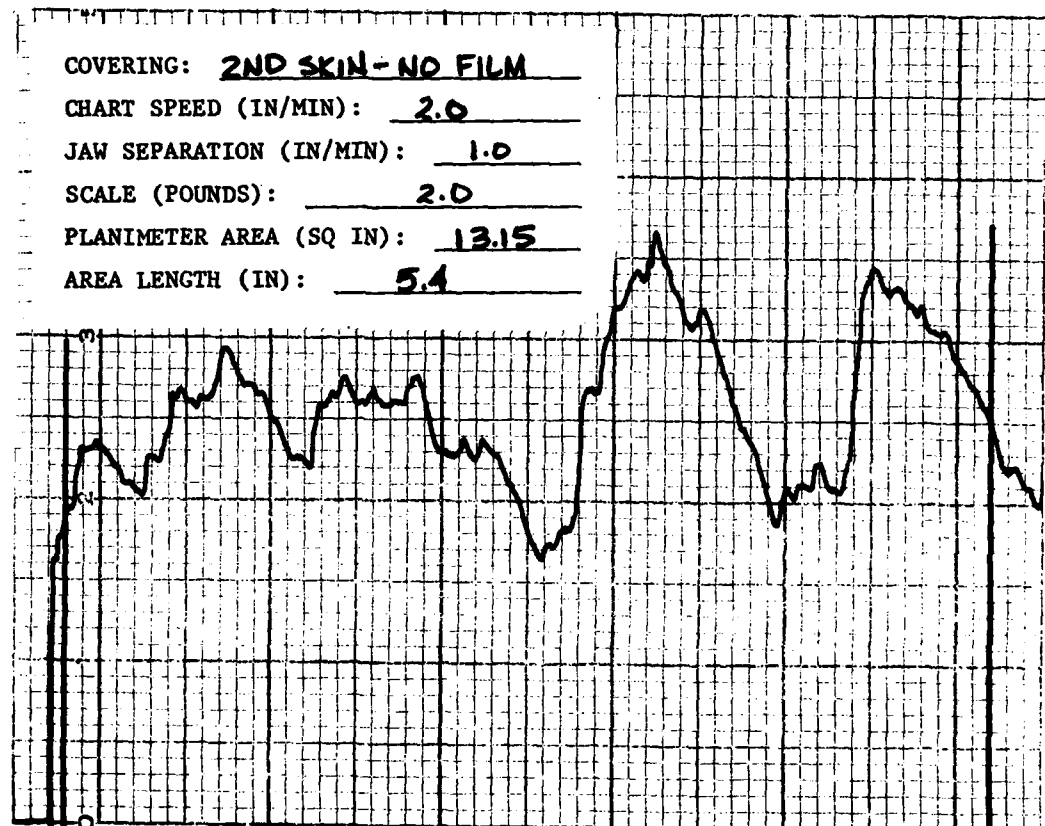
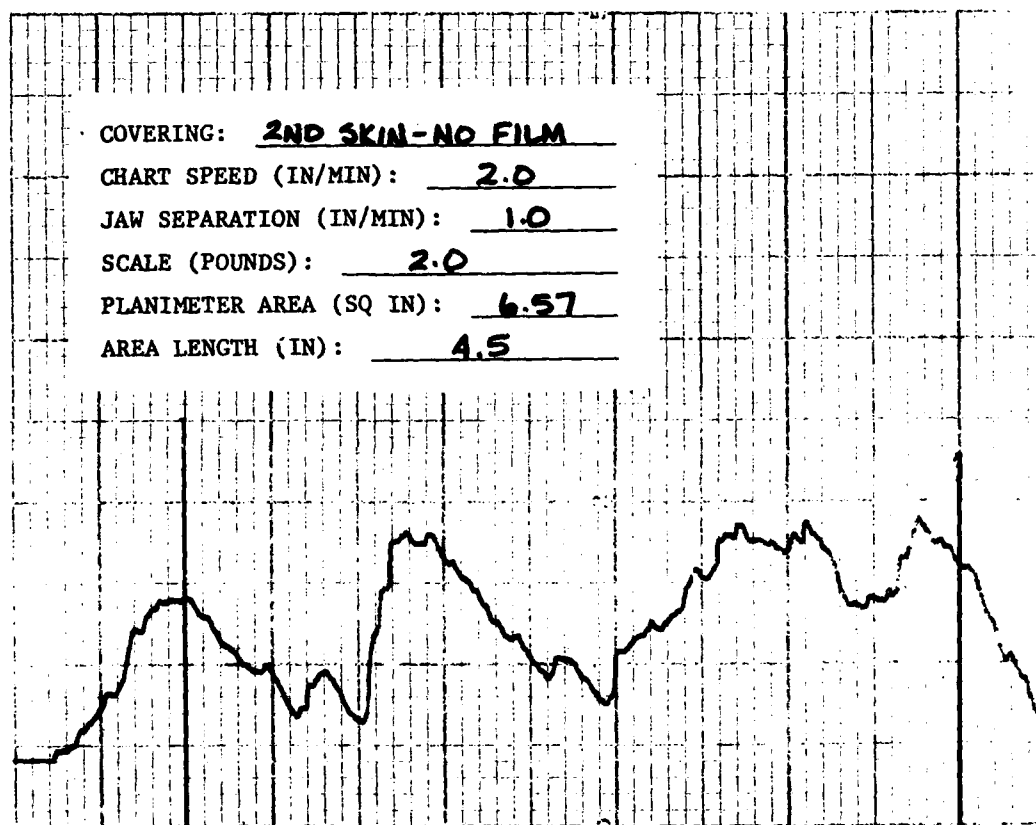


Figure 9.12

Second Skin (Film Off) Adherence Test
(Test No. 12 on Table 9.1)



and the results are shown in Table 9.1. Statistical analysis of the data is given in Table 9.2. The mean adherence of Biobrane was 11.2 g/cm, Second Skin with the film on was 5.4 g/cm, and Second Skin with the film off was 48.6 g/cm.

9.5 Discussion

These results, though not comprehensive, do show general differences between coverings. Second Skin with the film left on the exposed side had the lowest adherence. There was a good deal of fluid accumulation under the covering so there was not much hope of good adherence. It should be noted that Second Skin is not meant to be used in full excision wounds; it is to be used as a first-aid for minor cuts and burns.

Biobrane is a covering consisting of an ultra-thin membrane of poly-dimethylsiloxane mechanically bonded to a very fine knit, flexible nylon fabric. A non-toxic hypoallergenic mixture of highly-purified peptides derived from dermal collagen is bonded to the elastic membrane, providing a flexible covering. Biobrane is meant to be used on split-thickness donor sites, as well as on excised or debrided second and third degree burn sites as a temporary covering (2-4 weeks). We found Biobrane to be a superior covering compared to the Second Skin covering. The adherence was 11.2 g/cm, and there was no fluid accumulation under the dressing. These rats looked the healthiest. The covering draped well and provided a good covering, yet was easily removed without any tissue failure.

Second Skin used without the film on the exposed side had the highest adherence, 48.6 g/cm. The covering on this set of rats was extremely dry. When the covering was pulled off, there was a good deal of tissue failure--the muscles gave way before the covering came off. This was a poor covering as it was used.

Table 9.1

ADHERENCE CALCULATIONS FOR DATA

TEST NO.	COVERING	PLANIMETER AREA (in ²)	AREA LENGTH (in)	SCALE (lbs/in)	ADHERENCE (lb/in)	ADHERENCE (lb/cm)	COMMENTS
1	Biobrane	7.66	5.1	0.1	0.0834	14.3	some stretching of the bandage
2	Biobrane	11.12	8.4	0.1	0.0735	12.6	good pull, rat died
3	Biobrane	6.61	9.2	0.1	0.0399	6.8	
4	Inverted Biobrane	2.05	8.9	0.1	0.0128	2.19	the woven side was up, thus, not as much tissue adherence as 1-3
5	Second Skin Film On	5.30	8.3	0.1	0.0355	6.1	considerable pus
6	Second Skin Film On	3.78	7.0	0.1	0.0300	5.1	pus
7	Second Skin Film On	2.58	4.0	0.1	0.0358	6.1	pus
8	Second Skin Film On	1.87	4.2	0.1	0.0247	4.2	pus
9	Second Skin Film Off	5.10	3.5	0.5	0.4095	70.1	tissue failure, no pus
10	Second Skin Film Off	10.03	3.8	0.2	0.2930	50.2	tissue failure, some pus
11	Second Skin Film Off	13.15	5.4	0.2	0.2706	46.3	tissue failure
12	Second Skin Film Off	6.57	4.5	0.2	0.1622	27.8	some tissue failure

Notes:

- A) Chart speed in all cases was 2"/mm.
 B) Read speed in all cases was 1"/mm.
 C) Bandage width was 1.8".

Table 9.2
STATISTICAL ANALYSIS OF ADHERENCE DATA
(ALL NUMBERS IN g/cm)

SAMPLE	ADHERENCE DATA	MEAN (\bar{X})	STANDARD DEVIATION (S)	90% CONFIDENCE LIMITS*
Biobrane	14.3 12.6 6.8	11.2	3.9	11.2 ± 6.5
Second Skin Film On	6.1 5.1 6.1 4.2	5.4	0.9	5.4 ± 1.1
Second Skin Film Off	70.1 50.2 46.3 27.8	48.6	17.3	48.6 ± 20.3

*Defined by $\bar{X} \pm tS/\sqrt{N}$

Where: N = number of observations

S = standard deviation

t = correction factor (from Handbook of Chemistry and Physics, 41st ed.) (t = 2.92 and 2.35 for 2 and 3 degrees of freedom, respectively)

9.6 Conclusions

This series of rat tests quantitatively examined the adherence of burn coverings to the fully excised backs of rats. Some coverings were not available for testing; some coverings were not applicable to this method of testing. Biobrane gave the best drapability and had an adherence of 11.2 g/cm. Spenco Second Skin with and without film gave low and high adherence values, respectively, but it is not meant to be used in this manner.

9.7 References

Schmolka, Irving R. 1972. Artificial Skin I. Preparation and Properties of Pluronics F-127 Gels for Treatment of Burns. J. Biomed. Mater. Res. Vol. 6 p. 571-582.

Section 10

WIPE-ON COVERING EVALUATION IN PIGS

The purpose of these experiments was to quantitatively evaluate in miniature pigs a wipe-on covering developed for the Navy by Dynatech. The wipe-on, a solution of poly- ϵ -caprolactone (PCL), methyl acetate, methylene chloride, and triethyl citrate (a plasticizer), was developed under Contract No. N00014-73-C-0201. The pig study was carried out at Toxicol-Sisa, Inc., in Cambridge, Massachusetts, under the direction of Ellen Essigman, Ph.D.

The work was performed in two parts: a Pilot Study and a Main Study. The Pilot Study involved two pigs and was designed to:

- a) to determine an accurate and reproducible procedure for generating burn wounds in miniature pigs;
- b) to try out and evaluate several parameters for further studies designed to determine the efficacy of a "wipe-on" burn covering in miniature pigs; and
- c) to familiarize technical staff with procedures and techniques such as pig handling, pig anesthesia, burn generation, and application of a "wipe-on" burn covering.

That study resulted in: a method for anesthetizing the pigs during procedures; a method for restraining the animal during observation periods; a reproducible procedure for generating burns between second and third degree in severity; methods for grading the severity of the burn wound by histological and clinical observation; methods for measuring the microbial activity at the site; a technique for applying the wipe-on covering; and a method for measuring transepidermal water loss. These are discussed in detail in Toxicol-Sisa's Final and Supplementary Reports, entitled "Pilot Study for Evaluation of Synthetic Polymer "Wipe-on" Burn Covering in Miniature Pigs for Dynatech R/D Company, Cambridge, Massachusetts," and beginning on pages 103 and 159, respectively.

The Main Study used the methods developed in the Pilot Study to evaluate the wipe on covering in terms of microbial activity, transepidermal water loss, and clinical and histopathological examination. There were twelve sites evaluated on the pig's back. One was unburned and uncovered, one unburned and covered, five burned and uncovered, and five burned and covered. The procedures used in and results from this study are contained in Toxicol-Sisa's report "Main Study for Evaluation of Synthetic Polymer "Wipe-On" Burn Covering in the Miniature Pig for Dynatech R/D Company, Cambridge, Massachusetts," beginning on page 62.

The results from one of the pigs in the pilot study may be examined along with those from the main study. Four points deserve discussion:

- 1) In neither case did the wipe-on covering appear to have any positive or negative effect on the burn site, as evaluated by histopathological and clinical examination.
- 2) Microbial evaluation in the main study pig yielded a positive effect from the burn covering. That is, the covered sites had significantly less microbial activity than the untreated sites. There was no difference seen in the pilot study pig.
- 3) There was a significant positive effect on transepidermal water loss at 48 hours in the Main Study; the covered sites' evaporation rate was less than that of the uncovered sites. There was no difference in the pilot study pig.
- 4) The covering in the main study adhered much better than in the pilot study. The covering in the pilot study was easily removed in one piece, while in the main study the covering adhered tightly to both burned and unburned sites.

Thus, the results of the two studies are not well-defined, although two conclusions may be drawn, keeping in mind the limited data. First, when the wound covering adhered well, positive results were seen in terms of water loss and microbial activity. Second, neither study showed positive results when evaluated histopathologically. The fact that the well adhering covering in main study limited evaporation and microbial growth is encouraging; however, more study is needed before firm conclusions may be made.

Please Quote: TS 1181-1
DP-1/PCL

PILOT STUDY FOR EVALUATION OF SYNTHETIC
POLYMER "WIPE ON" BURN COVERING IN MINIATURE
PIGS FOR DYNATECH R/D COMPANY, CAMBRIDGE, MA
(DP-1/PCL)

Final Report

By

Ellen M. Essigmann, Ph.D.

December 14, 1981

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- Appendix II Clinical Observations on Burn Sites Produced in 10/7/81 Burn Generation Study Trial I Performed on Pig #336
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- Appendix IV Method For Calculating the Rate of Evaporation in Pig Skin
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- Diagram I Burn Diagram for 10/7/81 Burn Generation Study Trial I on Pig #336
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PILOT STUDY FOR EVALUATION OF SYNTHETIC POLYMER

"WIPE ON" BURN COVERING IN MINIATURE PIGS

Test Material: Poly-E-Caprolactone (#26341) in triethylcitrate, methylene chloride and methyl acetate

Toxicol-Sisa Ref. No.: TS 1181-1

Project: DP-1/PCL

Summary

A pilot study was performed for the purpose of developing and evaluating methods for performing an efficacy study on a "wipe on" burn dressing. The methods developed included a procedure for anesthetizing the pig during the burning procedure, a method for restraining the pig during the observation period of 48 hours, a method for generating burns which were reproducibly between second and third degree in severity, methods for grading the burn wounds at the clinical and histological level, methods for evaluating the microbial activity on the surface of the burn wound, a method for applying the "wipe on" dressing, and a method for measuring the rate of evaporation from the skin surface. The methods were described and evaluated and suggestions were made for improving the methodology in future burn studies. Preliminary data comparing uncovered versus covered wound sites in terms of the severity of the wound, rate of water loss and microbial activity was also generated. The evidence, based on a one pig study with four covered and four uncovered burns, does not support a significant difference between covered and uncovered wounds evaluated 48 hours after burning.

PILOT STUDY FOR EVALUATION OF SYNTHETIC POLYMER

"WIPE ON" BURN COVERING IN MINIATURE PIGS

Test Material: Poly-E-Caprolactone (#26341) in triethylcitrate,
methylene chloride and methylacetate

Toxicol-Sisa Reference No.: TS 1181-1

Project: DP-1/PCL

Overall Objectives

To determine an accurate and reproducible procedure for generating burn wounds in miniature pigs.

To try out and evaluate several parameters for further studies designed to determine the efficacy of a "wipe on" burn covering in miniature pigs.

To familiarize technical staff with procedures and techniques such as pig handling, pig anesthesia, burn generation and application of a "wipe on" burn covering.

Test Material

A clear, pale amber fluid in a clear glass, screw-capped jar labelled Dynatech #011280-1 was received from Dynatech R/D Co. on November 5, 1981. A small amount of the polymer appeared to have settled to the bottom of the jar. Approximately 300 ml of material was provided, along with two 1" wide natural bristle brushes to use in wiping on the dressing. The sample was designated TS 1181-1 and the project, DP-1/PCL.

The test material was reported by Dynatech to consist of 40g of Poly-E-Caprolactone (#26341) dissolved in a mixture of triethylcitrate, methylacetate and methylene chloride (Appendix I). This formulation differs from that specified in the Protocol DP-1/PCL. The formulation was changed in order to produce a solution of adequate viscosity.

Purity Analysis

No analyses of the sample material were performed by Toxicol-Sisa.

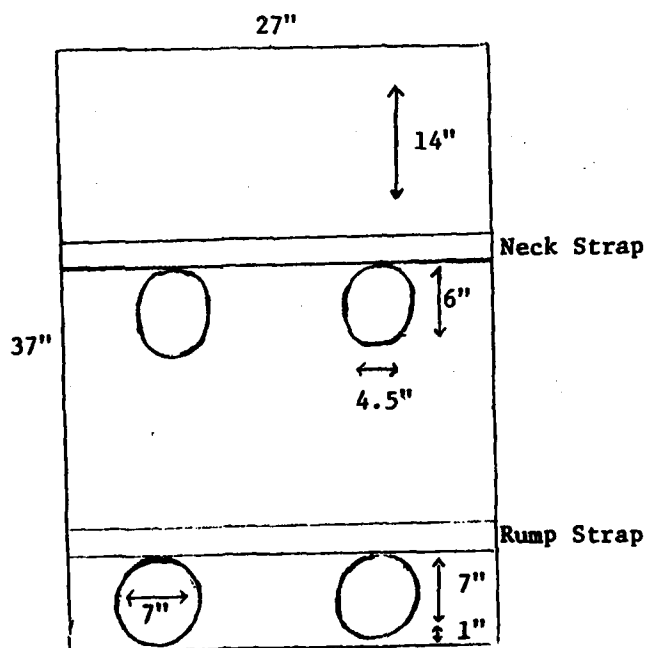
Animals

Female miniature pigs of the Pitman-Moore strain, aged 6 to 7 months and weighing between 15 and 18kgs, were supplied in one shipment by Vita Vet Laboratories, Inc., Marion, Indiana. The acclimation period was 14 days, during which time the pigs were subjected to an examination for clinical signs of disease by Dr. James Fox, D.V.M. The animals were housed in stainless steel dog metabolism cages under controlled temperature ($70 \pm 5^\circ\text{F}$), humidity (30 to 70%) and lighting conditions (12 hours light/12 hours dark). The pigs were identified by ear tag and cage card. Approximately 500 grams of chow (Respond 2000 dog food, Agway, Inc. Syracuse, NY), mixed with water, was provided twice daily. Water (approximately 2 liters) was provided three times a day. The pigs were weighed weekly during the first month of the study, and thereafter they were weighed prior to treatment. The pigs were allowed to exercise during the weighing period.

Materials

Pig Sling

A pig sling custom made by Bailey's Sportswear Inc., Boston, MA was constructed of heavy canvas with nylon lined leg holes and 1 1/2" wide heavy nylon straps with toothed buckles. The dimensions are given in the diagram below. The sling was attached to an angle iron frame using nylon rope threaded through a series of grommets set into the sling. The frame measured 18" wide, 59" long and 42" high.



Evaporometer

A hygrometer (hygro dynamics, Silver Spring, MD) fitted with a green humidity sensing probe (#733622) set within a plastic housing with a 25.81 cm² (2 x 2") opening and a volume of 0.213 liters was provided by Dynatech R/D, Inc.

Branding Irons

Three stainless steel branding irons with removable wood handles were provided by Dynatech R/D, Inc. The dimensions of each iron were 2" high by 2" wide by 2" deep. Each iron weighed approximately 1 kg. A set of weights totalling approximately 2 kg were added to each iron for some burn experiments.

Oil Baths

Glass crystallization dishes containing Molykote silicone fluid (Dow Corning Corp., Midland, MI) and electrically heated using a 10 foot coil of 1.02 ohms/foot wire connected to an autotransformer (Powerstat, Superior Electric Co., Bristol, CT).

ANESTHESIA STUDY

Objectives

- To obtain experience with the anesthesia procedure
- To determine the depth and duration of anesthesia and the length of the recovery period
- To prepare the pig's back for subsequent burn studies

Trial I: Pig #335

Experimental Design & Results

Pig #335, weighing 60 lbs., was used for this trial. Following a 24 hour fast, the pig was injected (i.m.) with a mixture of xylazine (3mg/lb.) and ketamine (5mg/lb.) The pig was then transferred to a canvas sling and monitored for 1 1/2 hours for respiratory rate and response to stimuli, including jabbing of the soft areas of the nose with a sharp object. Capillary refill time and pulse were not monitored. The initial dose resulted in insufficient analgesia and anesthesia and, therefore, was supplemented with an additional half dose (1.5mg/lb. of xylazine and 2.5mg/lb. of ketamine) approximately 40 minutes after the initial dose. Full anesthesia, as evidenced by a decreased and stable respiratory rate, lasted for approximately 20 minutes; full analgesia, as evidenced by the absence of response to pain stimuli, lasted for at least 30 minutes. The pig was returned to its cage 1 1/2 hours after the initial dose. It was provided with food and water approximately 4 hours after the last injection.

During the anesthesia trial, the pig's back was shaved with electric clippers, a depilatory cream (Nair) was applied, and the back was marked with indelible ink into 10 (2 x 2") squares, 5 on each side of the back bone, spaced 1" apart.

Conclusions

An initial dose of 3mg/lb. xylazine and 5mg/lb. ketamine, followed by a second half dose, resulted in approximately 20 minutes of full anesthetic and analgesic action, as determined by respiratory rate and response to mechanically induced pain stimuli. This was not felt to be sufficient for a burn generation trial. The pig appeared to be fully recovered within three to four hours of the final injection.

Trial II: Pig #336

Experimental Design

Pig #336, weighing 60 lbs., was used for this trial. The design of this trial was essentially the same as for Trial I with the following exceptions:

1. The full dose was increased from 3 to 4mg/lb. of xylazine and from 5 to 8mg/lb. of ketamine.
2. The pig's pulse was monitored.

3. Evaporometer readings were taken at site R2 approximately 30 minutes after Nair removal.

Results and Conclusions

The initial full dose of ketamine/xylazine was not sufficient to prevent spontaneous movement and response to dermal irritation caused by shaving and dipilatory cream application. This may have been due to the fact that part of the dose was inadvertently administered subcutaneously, rather than intramuscularly. An additional half dose (i.m.) resulted in full analgesic action for approximately 45 minutes. The duration of anesthetic action, evidenced by lack of spontaneous movement, decreased respiratory rate and pulse, was approximately 1 hour. Respiratory rate was unstable (9 to 72 breaths/minute) with irregular, shallow breathing interspersed with long periods of apnea. The pulse was relatively stable at 64 to 80 beats per minute. The pig was returned to its cage to recover. Full recovery occurred within 5 hours of the second injection.

The increased dose resulted in an increase in the strength and duration of the anesthetic and analgesic action with a prolonged recovery period. It was considered that the dose would be sufficient for providing anesthetic/analgesic action during subsequent burn studies.

The evaporometer readings resulted in a cumulative rate of water loss of 1×10^{-4} g/cm²sec. This data was not felt to be meaningful, as the readings were taken on skin which had recently been shaved and treated with a dipilatory cream.

Trial III: Pig #335

Experimental Design

This trial was performed using pig #335, weighing 65 lbs. The experimental design was identical to that used in Trial II, except that no supplemental doses were given, and the pulse rate was not monitored. The animal was allowed to recover fully while still in the sling in order to evaluate the effectiveness of the sling in restraining the animal over a long period of time.

Results and Conclusions

Approximately 25 minutes of full analgesic action and at least 35 minutes of anesthetic action were achieved. During this time, the respiratory rate was relatively stable at 43 to 49 breaths per minute. The pig appeared to be fully recovered within 2 hours of the injection. The sling proved effective in restraining the pig over a period of approximately 2.5 hours of intermittent struggling. The attachment of the sling to the frame was modified, following return of the pig to the cage, in order to facilitate feeding and watering the pig.

BURN GENERATION STUDY

Objectives

The major objective was to determine a procedure for generating wounds which are on the borderline of 2° to 3° in the dorsal skin of miniature pigs. A second objective was to perform preliminary evaluations of the evaporimeter apparatus and several methods of microbial assay of pig skin.

Another objective, determination of ³H-Proline dose, was not addressed as the Proline incorporation experiment was eliminated from the study at Dynatech's request.

Trial I

Experimental Design

Pig #336, weighing 64 lbs., was used for Trial I, initiated on October 7, 1981. One day prior to burn study initiation, the pig's back was reshaved following sedation with a 50mg oral dose of acepromazine. The pig was fasted for 24 hours prior to pre-burn anesthesia administration. The initial dose was 8mg/lb. ketamine and 4mg/lb. xylazine administered i.m. as a mixture. A second, 1/4 strength, injection of the mixture was required 35 minutes into the burn generation study.

Three stainless steel irons weighing approximately 1kg each were used for generating the burn wounds. The irons were heated in individual oil baths adjusted to 65°C. The burning procedure consisted of draining and wiping the iron over a 10 second period until it was relatively free of oil, applying the iron to the site for a designated time period, and replacing the iron in the oil bath to re-equilibrate.

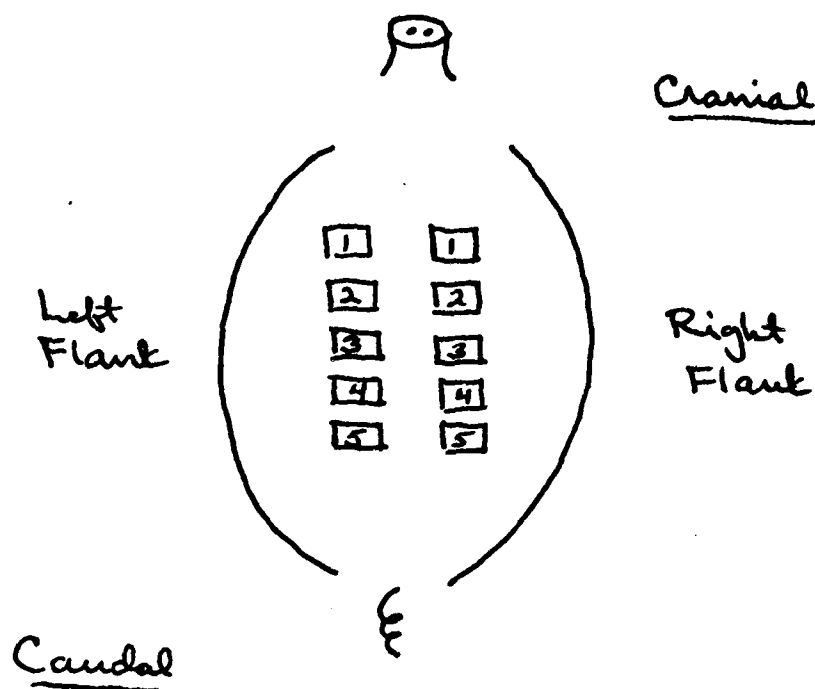
The sites were branded for time periods which varied from 10 to 45 seconds in duration (Diagram I, p.8). Light pressure was applied during burning of the first six sites. For the remaining sites (L4, L5, R4, R5), an additional 1000 grams of weight was added to the branding iron.

Approximately 1 hour after burning, the sites were examined visually and described. The pig was returned to the cage approximately eight hours after burning.

Forty-eight hours after burning, the pig was returned to the sling and site R5 (45 seconds with 2,000g of pressure per 2 inch square area) was biopsied for histopathological evaluation. The biopsy was taken from the most severe region of the burn site and was approximately 1 x 2cm in size. After removal of the biopsy sample, the wound site was filled with Gel-foam. The pig was not sacrificed but was returned to its cage. The biopsy sample was fixed in 10% neutral buffered formalin and prepared for histopathology examination as H & E stained sections.

DIAGRAM I

BURN DIAGRAM FOR 10/7/81 BURN GENERATION STUDY TRIAL I ON PIG #336



<u>Site</u>	<u>Duration (Seconds)</u> ¹
L1	10
L2	15
L3	25
L4	15 ²
L5	35 ²
R1	10
R2	15
R3	25
R4	25 ²
R5	45 ²

¹Branding iron heated to 65°C in oil bath²An additional 1000g of weight added to iron during branding

Results and Conclusions

The xylazine/ketamine injection given as an initial full strength dose followed by a quarter strength dose provided adequate anesthetic and analgesic action during the branding procedure.

The sling was felt to be ineffective in restraining the pig so that the pig had to be housed in its cage for the duration of the study.

The burn wounds, as they appeared approximately 1 hour after burning, are described in Appendix II. In general, the branding resulted in focal to diffuse severe erythema covering 40% or less of the burn site area. In most cases the areas showing a response to the branding iron application were located at the periphery of the sites where the edges of the branding iron had rested. The amount of weight applied during branding of four of the sites was increased in an attempt to achieve more contact between the iron and the skin. The most uniform and severe burn, based on visual examination at 48 hours post burning was that produced with 2kg of weight applied for 45 seconds (site R5). Approximately 60% of this site showed a response to the branding iron application. The response varied from extreme erythema to a whitening and blistering of the skin in the interior of the burn site.

At 48 hours after burning, the erythema in most of the sites had faded and it appeared that, with the exception of sites L5 and R5, the burns were first degree in severity. Sites L5 and R5 appeared to have areas which were 2° in severity. It was decided that R5 would be biopsied in order to get histological confirmation of this diagnosis. The remaining sites were left untouched so that they could be reused in a subsequent burn trial (Trial II).

The histopathology report on burn site R5 appears in Appendix III. It was characterized by full thickness necrosis of the epidermis with focal separation of the epidermis from the dermis and necrosis extending into the dermis a very short distance. Based on the classification formulated for the purpose of this study (Appendix III), this burn is second degree in severity.

The following conclusions were reached, based on the results of burn generation Trial I:

The burns produced in Trial I were not uniform and were not reproducible.

1. Additional weight should be added to the branding iron in order to achieve a more uniform burn within each site.

The burns produced were not severe enough.

2. The temperature of the branding iron should be increased and the range of branding duration times should be expanded in order to produce at least a few burns which are 2° bordering on 3° in severity.

The sling did not provide sufficient restraint of the pigs.

3. A second strap in front of the hind legs should be incorporated into the pig sling in order to adequately restrain the pigs.

Trial IIExperimental Design

This study was performed using pig #336, the same pig which was used for burn generation study, Trial I. At the time of the trial, 10/20/81, the pig weighed 71 lbs.

The procedure for anesthetizing and burning the pig are identical to those described for Trail I with the following exceptions:

1. The irons were heated to 70°C, rather than 65°C, and weighed approximately 3kg, rather than 1 or 2kg.
2. The duration of application varied from 10 to 80 seconds (Diagram II, p. 11). There were no duplicate sites.

As with Trial I, the pig was housed in its cage for most of the 48 hour study period. While under sedation with acepromizine (50mg), it was returned to the sling at 24 hours and then again at 48 hours for the purpose of burn evaluation, evaporometer readouts, microbial assay (48 hours only) and biopsy sampling (48 hours only).

Approximately 48 hours after burning, evaporometer readings were taken at four sites, three burned and one unburned, using the hygrometer. The housing was placed on the marked site and readings were taken at 10 second intervals over a 60 second period. Room temperature and humidity were noted. The method for calculating rate of evaporation in g/cm² sec. appears in Appendix IV .

Four of the burn sites, representing a range of branding iron application duration periods, were cultured 48 hours after burning. These were sites R1, L3, R4 and R5. The sites were sampled with sterile cotton tip applicator sticks. Aerobic bacteria were cultured using chocolate, blood McConky split plates and Rodac blood agar contact plates. A limited anaerobe screen included culturing on blood agar and thioglycolate plates.

All of the burn sites were biopsied 48 hours after burning. The biopsy samples taken were approximately 2 2/8 x 3/8" in size and were cut to include a portion of the subcutaneous fat layer. Biopsy samples were cut across the center of each site, starting within the unburned region cranial to the site and extending caudally into the unburned area on the opposite side. The samples were fixed in 10% neutral buffered formalin and prepared as H & E stained sections for histopathology examination.

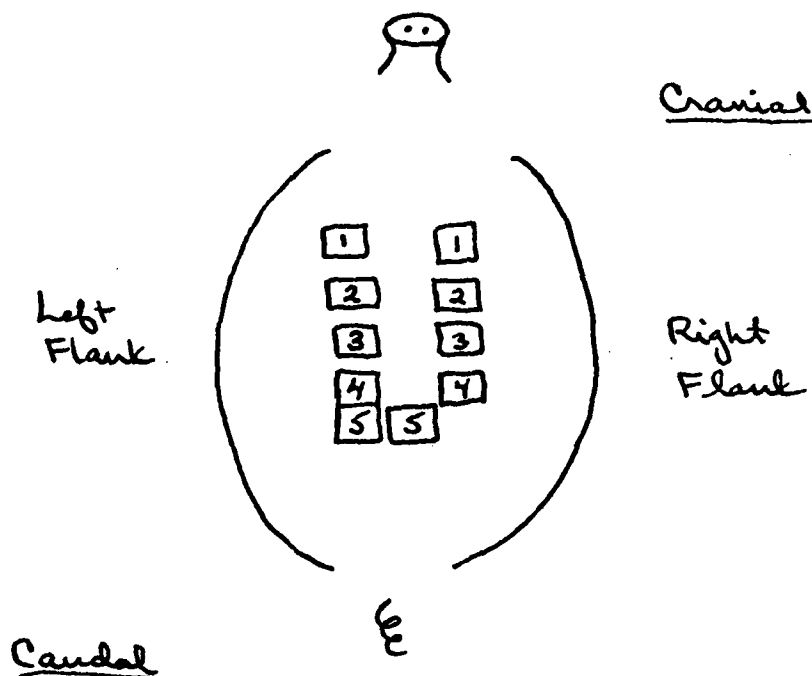
Results & ConclusionsClinical and Histopathological Data

Clinical observations made on the burn sites at 24 and 48 hours after burning appear in Appendix V . The nature and extent of the skin response to branding iron application varied both within individual sites and between sites.

In general, at least 40% of each site area showed a response to treatment which was appreciable at the clinical level.

DIAGRAM II

BURN DIAGRAM FOR 10/20/81 BURN GENERATION STUDY TRIAL II ON PIG #336



<u>Site</u>	<u>Duration (Seconds)*</u>
L1	30
L2	20
L3	35
L4	45
	65
R1	10
R2	25
R3	40
R4	55
R5	80

*Iron heated to 70°C in oil bath

Half of the sites showed a uniform response over 90% of their area (sites R2, R3, L1, L2, L3). Variability of response within the rest of the sites appeared to be related, at least in part, to uneven contact between the branding iron surface and the skin and perhaps to uneven application pressure. Sites burned for a relatively short duration (less than 30 seconds) were characterized by focal to diffuse severe erythema. Sites burned for 30 seconds or longer showed focal to diffuse whitening and occasional blistering of the skin adjacent to or within regions of severe erythema. The amount of whitening and blistering roughly correlated with the duration of treatment. Several of the sites showed areas of abraded skin which were produced when the pig was replaced in the sling for observation purposes.

Another relevant finding was that the thickness of the subcutaneous fat layer as observed at biopsy decreased from the cranial region of the back to the caudal region. This factor may have also had an effect on burn severity in the caudal region.

The results presented in the histopathology report (Appendix VI) provide evidence for intra- and inter- site variability of response to branding iron application. The range of response within and between sites varied from no appreciable reaction to damage characterized by partial to full thickness necrosis of the dermis. The rating system used to grade the burns is described in Appendix VII, and the histopathology results are tabulated in Table I on page 13. In Table I, each burn site was given an overall grade based on the predominant level of tissue damage present. In most cases, the most severe level of damage present was not the predominant level.

The least severe burn (R1) was produced with 10 seconds of heat application. This site showed predominantly no response, with small focal areas which were 1° or 1° bordering on 2° in severity. The most severe burn was produced with the longest period of branding iron application (80 seconds, site R5) and was predominantly 2° in nature with two large focal areas graded 2° bordering on 3° in severity.

Overall, the clinical and histopathological data shows an improvement over Trial I in terms of uniformity of response within each burn site. It also suggests that there is a loose correlation between duration of branding iron application and burn severity. The data, however, is not strong enough to enable the experimenter to select with confidence a branding temperature and duration for accurately reproducing burns which are 2° bordering on 3° in severity. Some of the variability and inconsistency observed in the present study may be due to variation in skin and subcutaneous fat thickness along the pig's dorsal surface. An appreciation for the effect of these factors on burn reproducibility within the same pig or between pigs will require further experimentation. Other factors which may effect reproducibility are the presence of varying amounts of dead skin debris on the pig's back and the existence of uneven pressure of branding iron application, both within a given site and between sites. These factors should be addressed in the experimental design of subsequent studies.

TABLE I

GRADING OF BURNS PRODUCED IN PIG #336 ON BURN GENERATION STUDY TRIAL II

PERFORMED 10/20/81

<u>Site</u>	<u>Duration of Branding</u> ¹	<u>Burn Severity</u>	
		<u>Overall Grade</u> ²	<u>Most Severe Grade</u> ³
R1	10	0	Focal 1°/2° ⁴
L2	20	1°/2°	Focal 1°/2°
R2	25	1°	Focal 1°/2°
L2	30	1°/2°	Focal 2°
L3	35	1°/2°	Focal 1°/2°
R3	40	1°/2°	Focal 2°
L4	45	2°	2°/3° ⁵
R4	55	2°	Focal 2°
L5	65	1°	Focal 2°
R5	80	2°	Diffuse 2°/3°

¹Duration of application of branding iron heated to 70°C in oil bath

²Grade given to characterize severity of burn which predominates in biopsy specimen

³Grade given to characterize most severe degree of tissue damage observed in the biopsy specimen

⁴1°/2° should be read as a burn which is first degree bordering on 2° in severity

⁵A single small focus of 2°/3° tissue damage was observed

Evaporometer Data

The evaporometer data produced from three burn sites and one unburned area is presented in Appendix VIII. The purpose of generating this data was to determine whether or not the evaporometer sensing element recommended by Dynatech (Green probe #733622) was capable of measuring the rate of water loss from both unburned and burned pig skin. The data indicates that the probe detected evaporation from untreated sites, burned sites which contained areas of mechanical abrasion and unabraded burn sites.

Microbial Assay (Appendix IX)

The limited anaerobic bacteria screen revealed the presence of obligately anaerobic bacteria, probably Clostridium spp., in all four sites. Aerobic bacteria cultured from all four sites included Staphylococcus epidermidis, Group D Streptococcus spp. and Staphylococcus spp. (not S. Aureus or S. epidermidis). One site (35 second duration) contained Moraxella spp. and another (55 second duration) contained Bacillus spp. and Klebsiella pneumoniae. All of these bacteria are commonly found on the skin surface of domestic animals. For all four sites, the contact plates produced colonies which were too numerous to count.

The results indicate that a method of serial dilution is needed for future studies requiring a quantitative assessment of the bacteria present in each site. Such an assay might focus on one commonly present microbe, such as Staphylococcus epidermidis, or it might be designed to determine the total number of aerobes, regardless of species, present on the skin surface of each site.

"WIPE ON" WOUND DRESSING STUDYObjectives

To perform a trial run of the proposed main study for evaluating the "wipe on" burn dressing.

Study Design

This study was performed using Pig #335, which weighed 90 lbs. at the initiation of the study (11/10/81).

The procedure for preparing and anesthetizing the pig was identical to that used for Burn Generation Study Trial II, except that the pig's back was lightly scrubbed with a nylon scrubbing pad 24 hours prior to burning, and only one full dose of ketamine/zyllazine was required.

The procedure for branding the pig was identical to that used for Burn Generation Study Trial II, with the following exceptions:

1. The irons were heated to 75°C, rather than 70°C.
2. The duration of branding for each burn site was 95 seconds.
3. The branding iron weight (3kg) was continuously shifted over the site during the application period in order to avoid uneven pressure distribution.

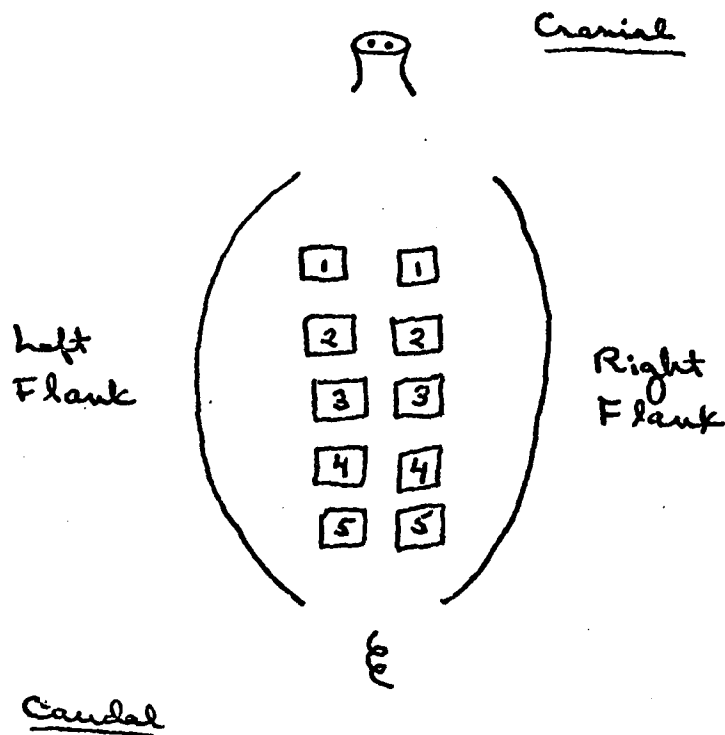
The "wipe on" dressing (TS 1181-1) was applied to half of the 10 sites as indicated in Diagram III, p 16. The method of dressing application was developed using the "wipe on" dressing, a 1" wide natural bristle brush and a piece of leather. Uniform coverage was obtained by soaking the brush in the dressing solution, lightly wiping the brush against the side of the jar and painting approximately half of the site in one even stroke. The brush was redipped for brushing the remaining half of the site. The "dry" brush was then used to re-wipe the site in order to more evenly distribute the still fluid dressing material and to extend the dressing coverage over a 1/4" wide border of adjacent normal skin. The dressing was applied approximately 10 minutes after burning each site.

The sites were described at 10 minutes after burning and again 48 hours after burning (just following removal of the "wipe on" dressing material). Evaporometer readings were taken on all sites at 4, 24 and 48 hours after burning. The method used was identical to that described for the Burn Generation Study Trial II, except that the 48 hour readings were taken for 3 minutes, rather than 1 minute.

The "wipe on" dressing was removed, using sterile forceps, as a single piece. All of the sites were described, photographed and then cultured using sterile cotton swabs dipped in Tryp-Soy Broth (Scott Laboratories, Fiskeville, RI). Two swabs were separately rolled over the entire surface of each site and then placed in approximately 1 ml of broth. The tubes were maintained at approximately 15°C during transportation to the microbiology lab at M.I.T. Six dilutions were prepared from each sample (10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} and 10^{-6}). Each dilution was plated onto 2 tryptic soy agar plates. Plates with between 30 and 300 colonies were counted; the average count from two plates was taken as the culture result.

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DIAGRAM III

BURN DIAGRAM FOR 11/10/81 "WIPE ON" DRESSING STUDY PERFORMED ON PIC #335 USING TS 1181-1



<u>Site</u>	<u>Thermal Treatment*</u>	<u>Dressing Treatment**</u>
L1	Unburned	Uncovered
L2	Burned	Covered
L3	Burned	Uncovered
L4	Burned	Covered
L5	Burned	Uncovered
R1	Unburned	Covered
R2	Burned	Uncovered
R3	Burned	Covered
R4	Burned	Uncovered
R5	Burned	Covered

*Branded for 95 seconds with an iron heated to 75°C in an oil bath

**"Wipe on" dressing TS 1181-1 applied using a 1" wide natural bristle brush

Biopsy samples were taken from each site as described for Burn Generation Study Trial II.

The pig was maintained within the sling for approximately 48 hours after burning. During this time it was offered its usual diet of chow and water twice daily. At 24 hours post branding, two impacted teeth were extracted from the pig's upper jaw, following an i.v. injection with Valium. Between 28 and 35 hours post branding, the pig was given approximately 1 gallon of orange juice (diluted 1:1 with water) by oral gavage. An i.v. dose of Acepromazine (3mg) was required in order to relax the pig during gavage.

Results & Conclusions

General

Following the shaving and marking of the pig's back twenty-four hours prior to burning (11/9/81), the sling frame turned over with the pig in it. As a result of this fall, the pig developed considerable edema, contusions and abrasions of the skin in the region of the marked burn sites. The location of two of the sites (L1 and L2) were shifted slightly in order to avoid these lesions. In other burn sites where it was not possible to completely avoid the lesions, the nature and location of the damage was described so that it would not be confused with damage related to subsequent burning.

During the first 8 hours following burning, the pig appeared to have tolerated the burning and restraint within the sling well. The animal ate and drank approximately 4 hours after burning and appeared to have an excellent appetite. On the following day the pig refused both food and water. A veterinary examination revealed the presence of inflamed and eroded gums associated with two impacted incisor teeth. The teeth were extracted and the animal was given a mixture of orange juice and water by oral gavage in order to prevent dehydration. The remainder of the study was uneventful with respect to husbandry considerations.

Clinical and Histopathological Data

The clinical observations made at 10 minutes and 48 hours after burning are presented in Appendix X. The appearance of the sites, both at 10 minutes and at 48 hours, suggested that more uniform burns developed for this experiment than for the previous experiment. For each site, the entire branding area showed a well defined response to the heat treatment, as compared to large areas of normal skin remaining within the burn areas of Pig #336. All locations showed diffuse whitening of the tissue over 100% of the 2 x 2" area with varying amounts of red/purple and/or yellow/brown mottling. There was no apparent effect of the burn covering on the clinical appearance of the burn wound or of the normal (unburned) skin site.

The histopathology report (Appendix XI) gave confirmation at the histological level of the intra- and inter-site burn uniformity observed clinically. The H + E sections showed full thickness coagulative necrosis of the epidermis, coagulative necrosis of the superficial dermis, separation of the epidermis from the dermis, congestion of the superficial dermal vessels and minimal signs of cellular invasion for all eight burn sites. Based on these observations, all of the burns were graded 2° in severity.

TABLE II

GRADING OF BURNS PRODUCED IN FIG #335 IN "WIPE ON" DRESSING STUDY PERFORMED 11/10/81

<u>Site</u>	<u>Treatment</u>	<u>Burn Grade</u> ¹	
		<u>Overall</u> ⁴	<u>Most Severe</u> ⁵
L1	Unburned, uncovered	0	0
R1	Unburned, covered ²	0	0
L2	Burned ³ , covered	2°	2°
R3	Burned ³ , covered	2°	2°
L4	Burned ³ , covered	2°	2°
R5	Burned ³ , covered	2°	2°
R2	Burned, uncovered	2°	2°
L3	Burned, uncovered	2°	2°
R4	Burned, uncovered	2°	2°
L5	Burned, uncovered	2°	2°

¹H + E section of biopsy sample evaluated using burn grading system presented in Appendix VII .

²Covered with "wipe on" burn dressing TS 1181-1.

³Burned with a 75°C branding iron for a duration of 95 seconds.

⁴Overall - the grade given for the degree of tissue damage which predominated in the biopsy sample.

⁵Most Severe - the grade given for the most severe degree of tissue damage present in the biopsy sample.

Additional histopathology findings were that in a few sites (L3, R2 and R3) there was congestion of the deeper vasculature of the dermis, but no corresponding congestion in the vessels of the dermal midzone. One interpretation of this finding is that the vascular supply immediately adjacent to the burn site was interrupted. This finding, coupled with the almost total absence of inflammatory cells within the burn sites, suggests that there may have been more extensive damage to the dermis than can be appreciated in an H + E section of tissue taken 48 hours after burning.

The focal areas of scab formation, epidermal necrosis, bacterial accumulation and inflammatory infiltrate reported for sites L1, L2 and L3 are related to tissue damage caused by the fall on 11/9/81.

There was no apparent effect of the wound dressing on the severity of the burn wound as determined by histopathology evaluation (Table II, p. 18). All of the H + E sections of the biopsy samples were graded 2°, both in terms of the predominant and the most severe degree of tissue damage.

Microbial Assay

The results of the microbial assay are presented in Appendix XII. The average number of bacteria cultured from covered burn sites was 6.13×10^4 versus 8.54×10^3 for uncovered burn sites. The average indicates an approximately 10-fold higher level of bacteria present in covered versus uncovered sites. However, there does not appear to be any significant ($P = 0.05$) relationship between the number of bacteria present and the treatment of the burn site (covered versus uncovered). The number of data points available for analysis (4 covered, burned plus 4 uncovered, burned) was relatively small, so that a real difference would have to be of great magnitude in order to be significant. The nature of the burn wounds may have had an effect on the microbial activity present. Severe coagulation necrosis may preclude an early (48 hours or less) infiltration of the dead tissue by opportunistic bacteria. There may also be a relationship between site location (as expressed in terms of relative distance from the cranial region of the pig's back) and number of bacteria present. The number of bacteria present appeared to have substantially decreased from the site one region to the site five region, regardless of burning or covering treatment.

Evaporometer Data

The evaporometer data appears in Appendix XIII. There was not enough data present to evaluate the effect of the dressing on the rate of evaporation from unburned skin. The cumulative rate of evaporation ($\text{g}/\text{cm}^2\text{sec}$) for burned, covered vs burned, uncovered at each time interval were compared using the t test for two means. At the three time intervals evaluated (4, 24 and 48 hours post burning), there was no significant difference ($p = 0.05$) between the rate of evaporation from burned, covered compared to burned, uncovered skin. The average cumulative rates of evaporation are as follows:

<u>Time</u>	<u>Treatment</u>	<u>Mean ($\text{g}/\text{cm}^2\text{sec}$)</u>	<u>Standard Error</u>
4 hrs	burned, covered	1.59×10^{-7}	1.59×10^{-7}
	burned, uncovered	3.18×10^{-6}	1.84×10^{-7}
24 hrs	burned, covered	1.27×10^{-6}	1.07×10^{-6}
	burned, uncovered	1.59×10^{-6}	1.05×10^{-6}
48 hrs	burned, covered	5.00×10^{-10}	5.00×10^{-10}
	burned, uncovered	7.92×10^{-7}	5.12×10^{-7}

PERSONNEL INVOLVED ON STUDY

Study Director:	Ellen M. Essigmann, Ph.D.
Director of Laboratory:	Francis J. Mecler, Sc.D.
Toxicologist:	Charles Nicholas James
Staff Veterinarian:	James Fox, D.V.M. - M.I.T. Christopher Newcomber, D.V.M. - M.I.T.
Veterinary Pathologist:	James Murphy, D.V.M. - M.I.T.
Microbiologist:	Joel Ackerman, M.S. - M.I.T.

REPORT SUBMISSION SHEET

Submitted by: Ellen M. Essigmann
Ellen M. Essigmann, Ph.D.
Study Director
Department of Toxicology

Reviewed by: Francis J. Meeker
Francis J. Meeker, Sc.D.
Department Head
Department of Toxicology

APPENDIX I

FORMULATION OF "WIPE ON" DRESSING DESIGNATED TS 1181-1¹

#26341 Poly-E-Caproletrone	40g
Tri Ethyl Citrate (Pfaltz & Bauer)	5.8 ml
Methylene Chloride (Howe & French)	60 ml
Methyl Acetate (Matheson Coleman & Bell)	240 ml

¹Prepared by Ms. Connie West, Dynatech R/D as Dynatech #011280-1

APPENDIX II

CLINICAL OBSERVATIONS ON BURN SITES PRODUCED IN 10/7/81 BURN GENERATION STUDY TRIAL I

PERFORMED ON FIG #336

1 Hour After Burning

<u>Site</u>	<u>Duration</u>	<u>Weight of Iron (kg)</u>	<u>Description</u>
L1	10	1	Severe erythema covering 40% of site area
L2	15	1	Severe erythema covering 30% of site area
L3	25	1	Severe focal to diffuse erythema covering 10% of site area
L4	15	2	Severe erythema covering 15% of site area
L5	35	2	Severe erythema covering 40% of site with whitening of skin in 10% of the site area
R1	10	1	Severe erythema covering 30% of the site area
R2	15	1	Focal to diffuse severe erythema covering 15% of site area with whitening of 5% of the site area
R3	25	1	Severe erythema covering 10% of site
R4	25	2	Focal to diffuse severe erythema covering 15% of site area with whitening of 5% of the site area
R5	45	2	Severe erythema covering 40% of the site with whitening of the skin over 20% of the site

48 Hours After Burning

<u>Site</u>	<u>Duration</u>	<u>Weight of Iron (kg)</u>	<u>Description</u>
L1	10	1	Normal
L2	15	1	Normal
L3	25	1	Normal
L4	15	2	Normal
L5	35	2	Erythema and eschar formation over <4% of site area
R1	10	1	Normal
R2	15	1	Normal
R3	25	1	Normal
R4	25	2	Normal
R5	45	2	Erythema over 40% of site area with whitening and mild blistering of skin over 20% of area

APPENDIX III

HISTOPATHOLOGY REPORT ON SITE R5, PIG #336, BURN GENERATION STUDY TRIAL I (10/7/81)

SISA - Pig Burn Study

Pig #1: Preliminary Investigation

Skin (Acc. 81-1120)

This section is characterized by the presence of diffuse necrosis of the epidermis with only a few small portions of the epidermis unaffected. The necrotic process of the epidermis extends only a short way into the opening of hair follicles. There is separation of the epidermis from the dermis at a few sites and evidence of necrosis extending into the dermis a very short distance in a few areas. The dermis is congested and there are perivascular accumulations of polymorphonuclear leukocytes, some of which have undergone necrosis. These changes in the dermis are limited to the proximal one-third of the dermis.

APPENDIX IV

METHOD FOR CALCULATING THE RATE OF EVAPORATION IN PIG SKIN

Interpretation of Hygrometer Readings:

1. Record time and dial reading on sheet provided.
2. Read % relative humidity (RH) off graph (sensing element 733622) at specified temperature and record on sheet.
3. Calculate ΔRH , the change in RH.
4. Using ΔRH , calculate the change in water presence:

$$\Delta M = \Delta RH \times \text{Water (g/liter)} \times \text{Volume (liter)}$$

Where: Water = Amount of Water in Saturated Air at Specified Temperature, Obtained from Table I

Volume = Volume of Plexiglass Housing, 0.213 liters

5. Calculate ΔT (sec).
6. Obtain Rate of Evaporation ($\text{g/cm}^2\text{sec}$):

$$\text{Rate (g/cm}^2\text{sec)} = \Delta M / (\Delta T \times \text{Area})$$

Where Area = 25.81 cm^2 , The Area of the Wound Site

7. Calculate cumulative ΔM , cumulative ΔT , and cumulative rate in similar means

TABLE I

Temperature (°C)	Water (g/l)
15	0.0135
16	0.0144
17	0.0154
18	0.0164
19	0.0174
20	0.0185
21	0.0197
22	0.0210
23	0.0222
24	0.0237
25	0.0251

APPENDIX V

CLINICAL OBSERVATIONS ON BURN SITES PRODUCED IN 10/20/81 BURN GENERATION STUDY

TRIAL II PERFORMED ON PIG #336

One Hour After Burning

<u>Site</u>	<u>Duration of Burn</u>	<u>Description</u>
L1	30	Uniform severe erythema over 100% of site area
L2	20	Severe erythema over 95% of site area
L3	35	Severe erythema over 100% of site area
L4	45	Severe erythema over 40% of site area; Diffuse whitening of tissue over 60% of site area
L5	65	Severe erythema and diffuse whitening of tissue, interspersed over 100% of site area
R1	10	Mild to severe erythema over 80% of site area
R2	25	Severe erythema over 100% of site area
R3	40	Severe erythema over 100% of site area
R4	55	Severe erythema interspersed with diffuse whitening of the skin approximately 90% of site area
R5	80	Severe erythema and whitening of the skin over 80% of the site

24 Hours After Burning

<u>Site</u>	<u>Duration of Burn</u>	<u>Description</u>
L1	30	Severe erythema and diffuse whitening of the skin affecting the entire site with focal epidermal abrasion produced during slinging of pig
L2	20	Patches of severe erythema covering 90% of site area
L3	35	Patches of severe erythema covering 90% of site area
L4	45	Severe erythema with whitening and blistering of the skin over 65% of site
L5	65	Severe erythema with whitening and blistering of skin over 80% of the site

24 Hours After Burning (cont.)

<u>Site</u>	<u>Duration of Burn</u>	<u>Description</u>
R1	10	Severe erythema over 40% of site area
R2	25	Severe erythema over 100% of site area
R3	40	Severe erythema and whitening of tissue over entire site with focal epidermal abrasion produced during slinging of pig
R4	55	Severe erythema, whitening and mild blistering of tissue over 70% of site
R5	80	Severe erythema over 80% of site with extreme whitening of tissue toward the center of the burn and focal epidermal abrasion produced during slinging of the pig

48 Hours After Burning

<u>Site</u>	<u>Duration of Burn</u>	<u>Description</u>
L1	30	Uniform severe erythema with focal epidermal abrasion due to slinging of pig
L2	20	Scattered foci of severe erythema over 60% of site area
L3	35	Severe erythema, focal to diffuse over 75% of site area with mild blistering
L4	45	Uniform severe erythema over 70% of site area with slight blistering and focal epidermal abrasion due to slinging of pig
L5	65	Uniform severe erythema and whitening of tissue over 60% of site with some clear exudate present
R1	10	Scattered foci of severe erythema over 20% of site area
R2	25	Focal to diffuse severe erythema over 85% of site with slight blistering
R3	40	Uniform severe erythema over 100% of site with slight whitening of skin and focal epidermal abrasion due to slinging of pig
R4	55	Focal to diffuse severe erythema with whitening of tissue over 65% of site
R5	80	Severe erythema with extreme whitening of tissue over 70% of site area with focal epidermal abrasion due to slinging of pig

HISTOPATHOLOGY REPORT ON BURN GENERATION STUDY TRIAL II ON PIG #336 (10/20/81)

L1

- 1 This section incorporates epidermis, dermis, a thick panniculus adiposus, cutaneous muscle and another layer of adipose tissue beneath this. Should future sections differ from this I will comment on it, otherwise assume they are the same.

This section is characterized by the presence of normal appearing epidermal tissue at one end of the section, and halfway across the section there is beginning necrosis of the epidermis which progresses to separation from the underlying dermis and in one area extensive necrosis that extends into the dermis with an associated polymorphonuclear cell infiltration. The area of separation of epidermis and dermis is filled with a mixture of proteinaceous fluid and polymorphonuclear leukocytes. The underlying dermis is congested with small leukocytic perivascular cuffs surrounding some vessels. These changes are limited to that portion of the dermis underlying the necrotic epidermis and only the proximal one-third of the dermis is affected.

- 2 The changes in this section are relatively uniform throughout the section. The epidermis has undergone coagulative necrosis and has separated from the underlying dermis. Small accumulations of polymorphonuclear leukocytes and small amounts of proteinaceous fluid are evident in this area of separation. The underlying proximal portion of the dermis is characterized by congested vessels with mild leukocytic perivascular cuffing. That portion of the dermis immediately adjacent to the epidermis appears to have undergone some degree of coagulative necrosis and a small leukocytic infiltrate is present in this portion.
- 3 This section is characterized by diffuse involvement of the epidermis, but there are more severe changes in the central portion of the section than are present at either extreme. The changes at the periphery are characterized by coagulative necrosis of the epidermis with beginning separation of the epidermis from the dermis and an infiltration of leukocytes and proteinaceous fluid. These changes become more severe one progresses towards the center of the section, and here the changes are characterized by complete loss of the epidermis with extensive necrosis extending into the underlying

L1-3 dermis with a polymorphonuclear cell infiltrate beneath
(cont) the area of coagulative necrosis of the dermis. Underlying
dermal vessels are congested and leukocytic perivascular
infiltrates are present in some areas.

- 4 The changes in this section are limited to coagulative
necrosis of the epidermis, separation of the epidermis from
the dermis, and a diffuse polymorphonuclear cell infiltra-
tion into the area of separation. A small portion of the
underlying dermis has also undergone necrosis, and vessels
in the proximal portion of the dermis are congested and
have some mild perivascular leukocytic cuffing. A small
portion of the epidermis associated with a hair follicle
is intact and has not undergone necrosis.
- 5 This section is characterized by uniform coagulative necrosis
of the epidermis over approximately half of the section and
the remaining half of the section has normal intact epidermis
that has not undergone necrosis. In the necrotic area there
is beginning separation of the epidermis from the dermis with
some hemorrhage and leukocytic infiltration into the area of
separation. A small portion of the underlying dermis has also
undergone necrosis. Mild vascular congestion in the proximal
portion of the dermis is evident in only a small portion of
the section.
- 6 This section is characterized by normal intact dermis and
epidermis with no evidence of necrosis, congestion, or leuko-
cytic infiltration.

L2

- 1 The epidermis and dermis are intact, there is no evidence of
necrosis, separation of epidermis and dermis, congestion or
leukocytic infiltration.
- 2 This section is characterized by the presence of two or three
small foci where the epidermis has undergone mild coagulative
necrosis and there is beginning separation of the epidermis
and dermis with congestion of underlying vessels and mild
hemorrhage into the areas of separation. The majority of the
epidermis and dermis show no signs of necrosis, congestion,
hemorrhage or leukocytic infiltration.

L2 (cont'd)

- 3 This section is characterized by the presence of focal areas of coagulative necrosis of the epidermis. In some areas the necrosis has not extended through the full thickness of the epidermis. Some of the foci are characterized by separation of the epidermis and dermis, and congestion of underlying vessels in the proximal portion of the dermis. Focal leukocytic infiltration is present in one area of separation between dermis and epidermis. A small area of necrosis of the epidermis is also associated with a mild leukocytic infiltration.

- 4 This section is characterized by one area of partial necrosis of the epidermis, and another area where the entire thickness of the epidermis has undergone necrosis and there is beginning separation of epidermis from the underlying dermis. Mild congestion of underlying dermal vessels is also present in this area with a mild perivascular leukocytic infiltrate. The remaining portions of the epidermis and dermis are unaffected.

- 5 This section is characterized by the presence of coagulative necrosis of the epidermis of the central portion of the section that affects approximately one-half of the section. There is beginning separation of dermis and epidermis with mild congestion of underlying dermal vasculature. The remaining portions of the section are unaffected.

- 6 There is no evidence of necrosis of the dermis, congestion of underlying dermal vessels or leukocytic infiltration in this section.

L3

- 1 There is no evidence of dermal or epidermal necrosis, congestion or leukocytic infiltration.

- 2 A singular small focus of epidermal necrosis is present at one extreme end of this section. The remaining 98% of the section is unaffected. The changes in the necrotic area are characterized by early separation of epidermis from underlying dermis and congestion of a few vessels in the proximal portion of the dermis.

L3 (cont'd)

- 3 This section is characterized by small focal areas of necrosis of the epidermis with mild separation of the epidermis from the underlying dermis and mild congestion of vessels in the underlying adjacent dermis. There is no evidence of hemorrhage or leukocytic infiltration associated with these foci.
- 4 This section is characterized by the presence of focal areas of necrosis of the epidermis. In most of the areas the necrosis extends through the complete layer of the epidermis; however, in some areas there is only necrosis of a portion of the thickness of the epidermis. Early separation of epidermis from underlying dermis is evident in a few areas, and there is mild congestion of vessels in the underlying dermis. Minimal evidence of leukocytic infiltration is present in any of these foci.
- 5 This section is characterized by the presence of focal areas of necrosis of the epidermis, affecting approximately one-half of the section, and other areas are unaffected. The affected areas are characterized by necrosis that extends either completely or partially through the epidermis with early separation of epidermis from the underlying dermis and congestion of vessels in the underlying dermis. Minimal evidence of leukocytic infiltration is associated with any of these foci.
- 6 This section is characterized by the presence of necrosis of the epidermis at one extreme end of the section and the remaining portion of this section is unaffected. The necrotic areas are characterized by the presence of early separation of the dermis from the epidermis and mild congestion and leukocytic infiltration of the underlying dermis.

L4

- 1 This section is characterized by the absence of any areas of necrosis affecting the epidermis or dermis. A singular small focus of mononuclear cell infiltration of the dermis is present that is probably unrelated to the experimental procedure.

L4 (cont'd)

- 2 This section is characterized by the presence of uniform diffuse necrosis of the epidermis extending the full length of the section, except for a very small portion at one end of the section. The lesion is characterized by total coagulative necrosis of the epidermis with necrosis extending into a superficial layer of the dermis. There is extensive separation of the dermis from the epidermis with an accumulation of proteinaceous fluid in some portions of the section. The vessels in the underlying dermis are congested and a mild leukocytic perivascular infiltrate is present throughout most of this section.

- 3 There appears to be some artifactual distortion of this section at both extremes of the sections. I will eliminate this portion from the description. A recut would be necessary to verify the changes that might be present in this area. The remaining portion of the section is characterized by extensive diffuse necrosis extending through the epidermis and into the proximal portion of the dermis. There is complete separation of the epidermis from the dermis throughout the section and extensive congestion of dermal vessels extending halfway through the dermis. Some leukocytic infiltration is present in the area of the separation, plus some proteinaceous fluid. A mild leukocytic infiltrate is present in the adjacent dermis and is concentrated in the vicinity of the congested vessels.

- 4 This section also has some artifactual distortion present at both ends of the section. The major portion of this section other than the area of artifact is characterized by extensive diffuse necrosis of the epidermis extending into the proximal portion of the underlying dermis. There is extensive separation of epidermis from dermis with a moderate polymorphonuclear cell infiltration into the area of separation. A moderate amount of proteinaceous fluid is also present in this zone of separation. Dermal vessels throughout the proximal one-half of the dermis are congested. Focal moderate polymorphonuclear cell infiltration is present in the proximal portion of the dermis.

- 5 This section is characterized by the presence of extensive artifactual distortion of the tissue, but it would appear that necrosis did extend through the epidermis and approximately halfway into the underlying dermis. This section should be recut if there is a need for more complete histopathologic evaluation.

L4 (cont'd)

- 6 This section is characterized by intact, unaffected epidermis and dermis throughout approximately three-quarters of the section. At one perimeter of the section there is coagulative necrosis of the epidermis, and this necrosis extends into the proximal portion of the underlying dermis. A mild degree of congestion is present in underlying dermal vessels, but there is minimal evidence of leukocytic infiltration of the area. Beginning separation of epidermis from dermis is evident in a few foci.

L5

- 1 A small focal area of necrosis of the epidermis is present in the center of this section that is characterized by beginning separation of the dermis from the overlying epidermis and mild congestion of vessels in the underlying dermis. The remaining portion of the section is unaffected.
- 2 A singular small focus of necrosis of the epidermis is present at one end of this section that is characterized by only partial necrosis of the epidermis with mild congestion of vessels in the underlying dermis and some early leukocytic infiltration into the necrotic portion of the epidermis.
- 3 This section is characterized by the presence of intact dermis and epidermis without evidence of necrosis, vascular congestion of the dermis or leukocytic infiltration.
- 4 This section is characterized by diffuse necrosis of the epidermis affecting all but a very small portion of epidermis at one extreme end of the section. There is early separation of epidermis from underlying dermis with a mild leukocytic infiltration into the area of separation in a few foci. Vessels in the proximal portion of the dermis are congested and have mild perivascular leukocytic infiltrates throughout major portions of this section.
- 5 This section is characterized by diffuse coagulative necrosis of almost the entire epidermis with focal areas of intact epidermis at one end of the section. Beginning separation of epidermis from dermis is evident throughout the section and the vasculature in the underlying dermis is congested with mild leukocytic perivascular infiltration associated with some vessels. Proteinaceous fluid is present in the area of separation in a portion of the section and there is some evidence of necrosis of the dermis in the zone immediately adjacent to the areas of separation.

L5 (cont'd)

- 6 This section is characterized by coagulative necrosis of approximately two-thirds of the epidermis with the necrosis extending through the full thickness of the epidermis and separation of the epidermis from the underlying dermis. The underlying dermal vessels in the proximal portion of the dermis are congested and a mild leukocytic infiltrate is present both in the dermis and in some areas of separation. Partial necrosis of the epidermis is evident at one focus and vessels in the underlying dermis in this area are also congested. The most proximal portion of the dermis in apposition to the areas of separation have also undergone necrosis.

R1

- 1 This section is characterized by intact epidermis and dermis with no evidence of necrosis, congestion or inflammation.
- 2 This section is characterized by the presence of focal partial necrosis of the epidermis in two small areas of epidermis and a third area of necrosis that extends completely through the thickness of the epidermis. A mild degree of congestion is evident in vessels of the dermis beneath the foci of necrosis and there is evidence of separation of epidermis from the dermis in the one area with full thickness necrosis. There is minimal evidence of leukocytic infiltration associated with the areas of necrosis.
- 3 There is no evidence of epidermal necrosis, nor of congestion in the underlying dermis or section.
- 4 There is no evidence of necrosis in either the dermis or epidermis, nor areas of congestion of dermal vessels nor any leukocytic infiltrate.
- 5 A singular very small focus of epidermal necrosis that involves only a third of the thickness of the epidermis is present. Minimal evidence of vascular congestion is evident in the underlying dermis and there is no evidence of leukocytic infiltration. A portion of epidermis is missing from this section at one end of the section and cannot be evaluated for evidence of necrosis. There is no evidence of congestion in the underlying dermis, however.

R1 (cont'd)

- 6 There is no evidence of necrosis of the epidermis or dermis in this section, nor are there foci of congestion of dermal vessels or evidence of leukocytic infiltration.

R2

- 1 There is artifactual destruction of this section at one end of the section, however the remaining portions of the section have no evidence of necrosis in the dermis or epidermis nor evidence of congestion or leukocytic infiltration.
- 2 This section is characterized by large areas of necrosis of the full thickness of the epidermis with focal areas of intact non-necrotic epidermis adjacent to the necrotic areas. The necrotic foci are characterized by early separation of the epidermis from the dermis with small accumulations of proteinaceous fluid and leukocytes in some of the areas of separation. The vessels of the dermis immediately under the zones of necrotic epidermis are congested and there is mild perivascular leukocytic infiltrate associated with these areas.
- 3 There appear to be large areas of necrosis and sloughing of the epidermis, but this is compounded by the presence of artifactual distortion of the tissue, and this section should be recut for complete evaluation.
- 4 This section is characterized by the presence of alternating foci of epidermal necrosis and intact epidermis. In some areas, the epidermal necrosis extends through the full thickness of the epidermis, in other areas only a portion of the epidermis has undergone necrosis. There is beginning separation of the epidermis from the dermis in some areas with early leukocytic infiltration of these areas. The vessels in the dermis immediately adjacent to the necrotic areas are congested and there is a mild perivascular leukocytic infiltrate associated with these vessels.
- 5 This section is characterized by the presence of small foci of epidermal necrosis at one end of the section. One of these foci has complete necrosis of the full thickness of the epidermis; the other focus has only partial necrosis of the epidermis. Beginning separation is present between the dermis and epidermis, and vessels in the dermis immediately beneath the necrotic areas are congested and a mild perivascular leukocytic infiltrate is associated with these vessels. Some leukocytic infiltration of the necrotic tissue is also evident.

R2

- 6 There is no evidence of necrosis in either the epidermis or dermis nor are there areas of congestion of dermal vessels nor evidence of leukocytic infiltration.

R3

- 1 There is no evidence of necrosis in either the epidermis or dermis and there is no evidence of congestion or leukocytic infiltration of the dermis.
- 2 This section is characterized by the presence of intact epidermis and dermis through approximately one-half of the section. The other half of the section is characterized by focal areas of necrosis of the epidermis interrupted by small areas of intact epidermis. The necrotic areas are characterized by full thickness necrosis throughout most of the necrotic zones with partial necrosis of the epidermis in one area. There is beginning separation of the epidermis from the dermis with a leukocytic infiltration into the areas of separation. The vasculature in the dermis immediately adjacent to the areas of necrosis is congested and a mild perivascular leukocytic infiltrate is associated with these areas. There are focal areas of necrosis that extend into the dermis immediately adjacent to the epidermis.
- 3 This section is characterized by the presence of a small focus of intact epidermis and dermis at both extremes of the sections with necrosis of the epidermis extending throughout the remainder of the section, and in some areas the necrosis extends deep into the underlying dermis. In the areas of separation of the dermis from the epidermis there is a moderate polymorphonuclear cell infiltrate associated with accumulations of proteinaceous fluid. In the area of necrosis into the underlying dermis the epidermis is completely destroyed and sloughed, and there is extensive polymorphonuclear cell infiltration at the junction of necrotic and normal dermal tissue. A moderate degree of congestion is present in the vessels of the dermis immediately adjacent to the necrotic areas with slight leukocytic perivascular infiltration associated with the congested vessels.

R3 (cont'd)

- 4 This section is characterized by a small zone of intact epidermis and dermis at one end of the section, and the remaining 90% of the section is characterized by necrosis of the epidermis. The necrotic areas are characterized by full thickness necrosis, separation of the dermis from the epidermis, and focal leukocytic infiltration and focal accumulations of proteinaceous fluid in the zones of separation. The vessels in the dermis immediately adjacent to the necrotic epidermis are congested and a mild perivascular leukocytic infiltration is associated with these vessels. Focal areas of necrosis extending into the dermis are present. These foci are small and the zone of necrosis extends for only a short distance.

- 5 This section is characterized by relatively diffuse areas of necrosis of the epidermis interrupted by small areas of intact epidermis and dermis. The areas of necrosis are characterized by full thickness necrosis of the epidermis, separation of dermis from epidermis, and congestion of the vessels in the dermis immediately adjacent to the necrotic areas. A mild degree of perivascular leukocytic infiltration is associated with these congested vessels. There is minimal evidence of necrosis extending into the underlying dermis, however focal areas of hemorrhage are present in the dermis immediately adjacent to the necrotic epidermis.

- 6 This section is characterized by the presence of intact epidermis and dermis with no evidence of congestion or leukocytic infiltration throughout approximately 95% of the section, but there is a focal area of partial thickness necrosis of the epidermis with congestion and focal hemorrhage in the underlying dermis at one end of the section.

R4

- 1 This section is characterized by the presence of intact epidermis and dermis throughout approximately 80% of the section with necrosis of the epidermis throughout the remainder of the section. The necrotic area is characterized by full thickness necrosis, separation of the epidermis from the dermis, and some leukocytic infiltration associated with accumulations of proteinaceous fluid in the areas of separation. There is a slight degree of congestion of vessels in the dermis immediately adjacent to the necrotic areas with mild perivascular leukocytic infiltration.

R4

- 2 This section is characterized by the presence of diffuse necrosis of the epidermis throughout the entire section with separation of the epidermis from the dermis and mild diffuse necrosis of the immediately underlying dermis throughout the major portion of the section. The vasculature of the dermis immediately underlying the zones of necrosis is congested and a mild degree of perivascular leukocytic infiltration is associated with these vessels.

- 3 This section is characterized by the presence of diffuse necrosis of the epidermis extending the full length of the section with separation of the dermis from the underlying dermis and extension of the necrosis for a short distance in the dermis throughout the full length of the section. The dermal vasculature immediately adjacent to the necrotic area is congested and a mild degree of perivascular leukocytic infiltration is associated with the congested vessels. There is minimal evidence of a leukocytic infiltrate into the areas of separation of the epidermis from the dermis.

- 4 This section is characterized by the presence of diffuse full thickness necrosis of the epidermis throughout the full length of the section with beginning separation of the epidermis from the dermis and accumulations of proteinaceous fluid and polymorphonuclear leukocytes in those areas of separation. The vasculature in the dermis immediately underlying the area of necrosis is congested and a mild perivascular leukocytic infiltration is associated with these congested vessels. There are focal areas of necrosis of the dermis immediately adjacent to the areas of separation.

- 5 This section is characterized by the presence of full thickness necrosis of the epidermis interrupted by areas of intact epidermis and dermis. The intact areas are small and constitute only a small portion of the epidermis. The necrotic foci are characterized by full thickness necrosis with separation of the epidermis from the dermis and some focal areas of necrosis of the underlying dermis. There is a mild leukocytic infiltrate into the area of separation in a few small foci. The dermal vessels immediately adjacent to the areas of necrosis are congested and a mild perivascular leukocytic infiltration is associated with some of these vessels.

R4 (cont'd)

- 6 This section is characterized by the presence of an intact epidermis and dermis throughout approximately 80% of the section with necrosis of the remaining portion of epidermis. The area of necrosis of the epidermis is characterized by full thickness necrosis throughout most of the affected area with beginning separation of the epidermis from the dermis and a mild leukocytic infiltrate into the area of separation. The dermal vessels immediately adjacent to the zone of necrosis are congested and a mild perivascular leukocytic infiltrate is associated with these areas.

R5

- 1 This section is characterized by the presence of an intact epidermis and dermis throughout 95% of the section with a small focus of necrosis of the dermis at one end of the section. The necrotic areas are characterized by full thickness necrosis of the epidermis throughout most of the area with no evidence of separation of epidermis from dermis and a few vessels in the underlying dermis are congested but there is no evidence of a leukocytic infiltrate or perivascular cuffing.
- 2 This section is characterized by the presence of severe diffuse necrosis throughout the full length of the section. The necrotic zone extends through the full thickness of the epidermis into the underlying dermis for a short distance. In some areas the epidermis is sloughed; in other areas there is separation of the epidermis from the underlying dermis and at one end of the section there is extensive necrosis extending deep into the dermis almost to the level of the panniculus adiposus. Extensive congestion and hemorrhage is present in this area of deep necrosis. There is a suggestion of artifactual distortion associated with this section and it should be recut for complete evaluation.
- 3 This section is characterized by severe diffuse necrosis extending the full length of the section. The necrosis extends through the full thickness of the epidermis and a short distance into the underlying dermis throughout about half of the section. The remaining portion of the section has necrosis that appears to extend deep into the dermis almost to the level of the panniculus adiposus. There appears to be extensive serum accumulation on the surface of the skin and extensive sloughing of the epidermis over a large area. There is minimal evidence of leukocytic infiltration of the zone of separation of the epidermis from the dermis and no evidence of proteinaceous fluid. The

R5-3 (cont'd)

vessels in the dermis immediately adjacent to the necrotic area are congested and there is a slight perivascular mononuclear cell infiltrate associated with these congested vessels. A moderate leukocytic infiltration is associated with a hair shaft that is in the area of necrosis.

- 4 This section is characterized by the presence of severe diffuse necrosis throughout the full length of the section with extension of the necrosis through the full thickness of the epidermis for a short distance into the underlying dermis. The epidermis has separated from the dermis over a large portion of the section and accumulations of proteinaceous fluid are present in the area of separation. The vasculature in the dermis immediately adjacent to and deep to the necrotic area are congested and a mild perivascular leukocytic infiltrate is associated with these vessels.
- 5 This section is characterized by severe diffuse necrosis of the epidermis with extension of the necrosis through the full thickness of the epidermis and for a short distance into the underlying dermis. The epidermis is separated from the dermis throughout the entire length of this section and there are accumulations of proteinaceous fluid and leukocytes in the areas of separation. The vasculature in the dermis immediately adjacent to the areas of necrosis and also vessels deep in the dermis are congested. A mild perivascular leukocytic infiltrate is associated with those vessels close to the areas of necrosis. Large areas of hemorrhage are also present in the dermis associated with hair shafts and other skin adnexa.
- 6 This section is characterized by the presence of intact epidermis and dermis throughout approximately two-thirds of the width of the section with extensive necrosis of the epidermis and dermis in the remaining portions of the section. Most of the epidermis has sloughed and the necrotic zone extends deep into the underlying dermis. A zone of both intact and necrotic polymorphonuclear leukocytes are present in the area of junction of necrotic and normal dermal tissue. Focal areas of hemorrhage are present both in the necrotic area and normal tissue adjacent to the zones of necrosis. Accumulations of polymorphonuclear leukocytes, proteinaceous fluid and necrotic cell debris are on the surface of the skin in the area of severe necrosis. A recut is indicated.

APPENDIX VII

GRADING SYSTEM FOR THE HISTOPATHOLOGICAL EVALUATION OF THERMAL BURNS IN THE SKIN
OF MINIATURE PIGS¹

<u>Grade</u>	<u>Description</u>
0	Normal skin
1°	a) Partial or complete necrosis of the epidermis b) Congestion of the superficial dermal vessels
1°/2° ²	a) Same as above b) Same as above c) Separation of the epidermis from the dermis d) Exudation of leukocytes and proteinaceous fluid into area of separation
2°	a) Same as above b) Same as above c) Same as above d) Same as above e) Necrosis of the dermis at the junction of the dermis and epidermis (papillary region)
2°/3° ³	a) Same as above b) Same as above c) Same as above d) Same as above e) Same as above f) Dermal necrosis that extends beyond the papillary region but involves less than the full thickness of the dermal layer
3°	a) Same as above b) Same as above c) Same as above d) Same as above

e) Same as above

f) Full thickness dermal necrosis

¹Based on a modification of the burn classification systems discussed by Rudowski (Burn Therapy and Research, John Hopkins Union Press, Baltimore, 1976).

²1°/2° indicates a burn which is 1° bordering on 2° in severity.

³2°/3° indicates a burn which is 2° bordering on 3° in severity.

APPENDIX VIII

EVAPOROMETER DATA FOR BURN GENERATION STUDY TRIAL II ON FIG #336

<u>Site Treatment</u>	<u>Cumulative Rate of Evaporation (g/cm²sec.)</u>
Unburned	3.82×10^{-6}
Burned	2.35×10^{-5}
Burned	1.46×10^{-5}
Burned	3.94×10^{-5}

APPENDIX IX

MICROBIOLOGY REPORT ON BURN GENERATION STUDY - TRIAL II ON PIG #336

<u>Site</u>	<u>Burn Grading</u>		<u>Obligately</u>		<u>Quantitative Assay</u>
	<u>Overall</u>	<u>Most Severe</u>	<u>Aerobic Bacteria</u>	<u>Anaerobic Bacteria</u>	<u>(Contact Plate)</u>
R1	0	Focal 1°/2°	<u>Staphylococcus epidermidis</u> <u>Staphylococcus spp.</u> ¹ <u>Streptococcus spp.</u> Group D	Present ²	TNTC ³
L3	1°/2°	Focal 1°/2°	<u>Staphylococcus epidermidis</u> <u>Staphylococcus spp.</u> ¹ <u>Streptococcus spp.</u> Group D <u>Moraxella spp.</u>	Present ²	TNTC ³
R4	2°	Focal 2°	<u>Staphylococcus epidermidis</u> <u>Staphylococcus spp.</u> ¹ <u>Streptococcus spp.</u> <u>Bacillus spp.</u> <u>Klebsiella pneumoniae</u>	Present ²	TNTC ³
R5	2°	Diffuse 2°/3°	<u>Staphylococcus epidermidis</u> <u>Staphylococcus spp.</u> ¹ <u>Streptococcus spp.</u>	Present ²	TNTC ³

¹Not S. aureus or S. epidermidis

²Probably Clostridium spp.

³Too numerous to count; greater than 200 colonies per 16cm²

APPENDIX X

CLINICAL OBSERVATIONS ON BURN SITES PRODUCED 11/10/81 FOR "WIPE ON" DRESSING STUDY
PERFORMED ON PIG #335

10 Minutes After Burning

<u>Site</u>	<u>Burn</u>	<u>Dressing</u>	<u>Description</u>
L1	Unburned	Uncovered	Normal with exception of mild abrasion due to fall on 11/9/81
L2	Burned	Covered	65% uniformly whitened in appearance, 35% severe erythema; laceration, edema and erythema over 20% of area in left caudal corner of site due to 11/9/81 fall
L3	Burned	Uncovered	100% uniformly white with diffuse, severe erythema; a bruise, resulting from the 11/9/81 fall, covers approximately 15% of the left cranial corner of the site
L4	Burned	Covered	15% of site area uniform white; 85% of site area severe erythema with an area (10%) of diffuse white
L5	Burned	Uncovered	Severe erythema with diffuse whitening of tissues over 70% of site area; uniform whitening of tissue over 30% of area
R1	Unburned	Covered	Normal except for some mild abrasion due to fall on 11/9/81
R2	Burned	Uncovered	Uniform white with diffuse erythema
R3	Burned	Covered	Uniform white with diffuse erythema
R4	Burned	Uncovered	Uniform whitening of tissue over 95% of site with diffuse erythema covering 40% of this region; 5% of site characterized by uniform erythema only
R5	Burned	Covered	95% of site characterized by severe erythema with diffuse whitening of tissue; 5% of area characterized by uniform whitening of tissue

48 Hours After Burning

<u>Site</u>	<u>Burn</u>	<u>Dressing</u>	<u>Description</u>
L1	Unburned	Uncovered	Some abrasion scabs present due to fall on 11/9/81 (less than 5% of site)
L2	Burned	Covered	Whitened tissue with mottled yellow/brown (35%), severe erythema with uniform whitening of tissue (65%)
L3	Burned	Uncovered	Whitened tissue uniformly mottled yellow/brown with a few foci of purple (90% of area); scab due to fall on 11/9/81 covering 10% of area
L4	Burned	Covered	Whitened tissue mottled with purple foci (45% of area); whitened tissue with yellow/brown mottling (40% of area); uniformly whitened tissue covering 15% of area
L5	Burned	Uncovered	Whitened tissue with yellow/brown mottling (40% of area); whitened tissue with purple mottling (55% of area); uniformly whitened tissue (5% of area)
R1	Unburned	Covered	Scab due to 11/9/81 fall covering less than 5% of area
R2	Burned	Uncovered	Whitened tissue mottled with yellow/brown covering 90% of area; uniformly whitened tissue covering 10% of area
R3	Burned	Covered	Whitened tissue mottled with yellow/brown covering 95% of area; uniform whitening of tissue covering 5% of area
R4	Burned	Uncovered	Yellow/white tissue with brown mottling covering 70% of area; yellow/white tissue covering 25% of area; uniform whitening of tissue covering 5% of area
R5	Burned	Covered	65% of area characterized by red/purple and white mottling; 30% of area characterized by yellow/brown and white mottling; 5% uniform whitening of tissue

APPENDIX XI

HISTOPATHOLOGY REPORT ON "WIPE ON" DRESSING STUDY PERFORMED 11/10/81 ON PIG #335

Pilot Study

INTRODUCTION

Each slide submitted for evaluation contains two sections which represent the full cross section of each burn site with a small portion of normal skin at each perimeter. The skin sections are characterized by the presence of epidermis, dermis and a portion of the panniculus adiposis. The report of the histopathologic appearance of each of these two sections will be treated as though it were a single continuous section.

Section L1

This section is characterized by the presence of an intact epidermis throughout the entire length of the skin section with the exception of two small foci of necrosis near the center of the burn site and an additional small focus of necrosis near the perimeter of the burn site. The focus of necrosis located at the perimeter of the burn site is characterized by focal destruction of the epidermis and a small portion of the underlying dermis. A scab formation is present on the overlying surface that is composed of necrotic cell debris, proteinaceous fluid and large numbers of coccoid bacteria. A moderate number of polymorphonuclear leukocytes are present in the dermis and epidermis at the perimeter of the focus of necrosis. The two small foci of necrosis near the center of the burn site are similar in appearance and consist of focal necrosis of the epidermis extending superficially into the papillary portion of the dermis. Scab formation composed of necrotic cell debris and proteinaceous fluid with minimal evidence of bacteria is present on the overlying surface. A moderate number of polymorphonuclear leukocytes are present in the underlying epidermis and dermis at the perimeter of the necrotic area.

Section L2

This section is characterized by the presence of intact epidermis at both extremes of the tissue section peripheral to the burn site. The remaining portion of the section is characterized by diffuse coagulative necrosis of the epidermis which also extends for a short distance into the underlying papillary portion of the dermis. Extensive separation of epidermis from dermis is present in some areas, but some of this may represent an artifact of sectioning. Accumulations of proteinaceous fluid, a few leukocytes and a few red blood cells are present in the separation space between the epidermis and

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Section L2 (cont'd)

dermis in the central portion of the skin section. The dermal vasculature beneath this zone of separation is congested with focal perivascular hemorrhage. There is almost no evidence of cellular invasion of the burn site in this section of tissue.

Section L3

This section is characterized by the presence of intact epidermis and dermis at one extreme of the section, whereas at the other extreme of the section there is a focal area of necrosis of the epidermis that extends into the underlying dermis in the center of a portion of normal appearing, unaffected epidermis and dermis. The major portion of this section associated with the burn site is characterized by diffuse coagulative necrosis of the epidermis which also extends into the underlying papillary portion of the dermis. Extensive separation of the epidermis from the dermis is present throughout the section, but most of this appears to be an artifact of sectioning. Small accumulations of proteinaceous fluid and leukocytes are present in the zone of separation between the epidermis and dermis at the perimeter of the burn site at the junction of necrotic epidermis and normal intact epidermis. The underlying dermal vasculature in this area is congested. There is no evidence of congestion of vessels in the dermis of the central portion of the burn site, except in deeper vessels near the junction with the panniculus adiposis. This finding suggests an interruption of vascular supply to the dermis immediately adjacent to the burn site. There is also no evidence of leukocytic infiltration of those tissues associated with the burn site. The exception to this is that portion of tissue at the perimeter of the burn adjacent to normal tissue. The focal area of necrosis mentioned earlier is characterized by necrosis of the epidermis extending into the underlying papillary portion of the dermis, with scab formation on the overlying surface composed of necrotic cell debris, blood and proteinaceous fluid, and a polymorphonuclear cell infiltrate in the dermis and epidermis adjacent to the area of necrosis.

Section L4

This section is characterized by the presence of a zone of intact epidermis and dermis at both ends of the section with diffuse coagulative necrosis of the epidermis and underlying papillary portion of the dermis throughout the remainder of the section. The underlying dermal vessels are congested throughout the section in the region of dermal and epidermal necrosis, and evidence of congestion is present in the vessels deep in the

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Section L4 (cont'd)

dermis. Extensive separation of epidermis from dermis is present throughout the section in the area of necrosis, but some of this appears to be an artifact of sectioning. Focal areas of separation with an infiltrate of proteinaceous fluid into the zone throughout the section. Several vessels deep in the dermis in the central portion of the section contain fibrin thrombi. Extensive artifactual distortion of one of the two sections of skin precludes a critical evaluation of tissue changes in this section.

Section L5

This section is characterized by intact epidermis and dermis at the perimeter of the section with diffuse coagulative necrosis of the epidermis throughout the remaining portion of the section in the area of the burn. There is minimal evidence to suggest extension of necrosis into the underlying papillary portion of the dermis. A moderate degree of congestion is present in the dermal vessels immediately beneath the epidermis throughout the section, with the exception of the perimeters. Multiple small foci of separation of epidermis from the dermis are present with accumulations of proteinaceous fluid within these areas of separation. In addition, multiple small foci of separation between layers of cells in the epidermis with accumulations of proteinaceous fluid within these foci are present throughout the section. The majority of these contain both proteinaceous fluid and accumulations of red blood cells and the majority of them are located immediately beneath the cornified cell layer of the epidermis. Occasional small perivascular accumulations of leukocytes are present around small congested capillaries in the papillary portion of the epidermis.

Section R1

This section is characterized by the presence of intact epidermis and dermis throughout the entire length of the section with the exception of a singular small focus of necrosis of the epidermis which extends slightly into the underlying dermis, slightly to one side of the center of the burn site. This focus is characterized by the presence of scab formation on the overlying surface composed of a mixture of necrotic cell debris and proteinaceous fluid with total necrosis of the underlying epidermis and the papillary portion of the dermis. A slight polymorphonuclear cell infiltrate is present in the dermal tissue immediately adjacent to the focus of necrosis. Two additional small focal accumulations of necrotic cell debris are present in the keratin layer of the epidermis immediately adjacent to this area of necrosis

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Section R1 (cont'd)

described. There is minimal evidence of congestion of dermal vessels in the papillary portion of the dermis and minimal evidence of leukocytic infiltration except at the singular small focal area of necrosis.

Section R2

This section is characterized by the presence of intact epidermis and dermis at the periphery of the sections with extensive diffuse necrosis of the epidermis which extends into the underlying papillary portion of the dermis for a short distance. Multiple foci of separation of the epidermis from the dermis are present, however many of these appear to be artifacts of sectioning. Focal areas of separation of epidermis from dermis with an infiltration of proteinaceous fluid are present. Extensive artifactual distortion of one of the two sections of skin precludes a critical evaluation of this portion of the section. A moderate degree of congestion is present in capillaries in the dermis immediately adjacent to the epidermis and also is present in vessels deep in the dermis. Minimal evidence of leukocytic infiltration is evident in the dermis adjacent to the burn site.

Section R3

This burn site is characterized by the presence of intact epidermis and dermis at the perimeter of the sections with extensive coagulative necrosis of the epidermis extending into the underlying papillary portion of the dermis for a short distance throughout the remaining portion of the section. Extensive artifactual distortion is present in half of the section which precludes a critical evaluation of this tissue. Extensive areas of separation of epidermis from dermis are present throughout the section, however, many of these areas appear to be artifacts of sectioning. Numerous small focal areas of separation of epidermis from dermis with an infiltration of proteinaceous fluid are present, and a few small focal areas of separation between layers of cells in the epidermis proper with an infiltration of proteinaceous fluid into the space formed are also present. Dermal vessels immediately adjacent to the epidermis are congested throughout a major portion of the section and small perivascular aggregates of leukocytes are present around some of these vessels in a portion of the section. A moderate degree of congestion is also evident in vessels deep in the dermis.


Pilot Study
Page 5

Section R4

This burn site is characterized by the presence of intact epidermis and dermis at the perimeter of the section with extensive diffuse coagulative necrosis of the epidermis throughout the remainder of the section. Mild focal areas of coagulative necrosis are evident in the papillary portion of the dermis that is most evident near the central portion of the section. Multiple small focal areas of separation of epidermis from dermis are present throughout the portions of the section where necrosis has occurred, and some of these separations contain accumulations of proteinaceous fluid, occasionally a few red blood cells, and a few leukocytes. The dermal vessels in the papillary portion of the dermis are congested and mild perivascular leukocytic infiltrates are present in a few small foci. A few small foci of separation of epidermal cells with accumulations of proteinaceous fluid in the spaces formed are also present.

Section R5

This burn site is characterized by the presence of intact epidermis and dermis at the peripheral ends of the section with extensive diffuse coagulative necrosis that extends through the epidermis into the underlying papillary portion of the dermis for a short distance. A moderate degree of congestion is present in the dermal vessels adjacent to the necrotic tissue and numerous small foci of separation of epidermis from dermis with accumulations of proteinaceous fluid or red blood cells in the area of separation are present throughout the section. Some areas of separation of epidermis from dermis are thought to be artifacts of sectioning. A small focal area of polymorphonuclear cell infiltration adjacent to the area of necrosis is present at the perimeter of the necrotic zone at one end of the section.


James C. Murphy, DVM, PhD
Veterinary Pathologist

APPENDIX XII

MICROBIOLOGY REPORT ON "WIPE ON" DRESSING STUDY (11/10/81) PERFORMED ON PIG #335

<u>Site</u>	<u>Treatment</u>	<u>Number of Colonies</u> ¹
L1	Unburned, uncovered	1.77×10^5
R1	Unburned, covered ²	1.11×10^6
L2	Burned ³ , covered	1.92×10^5
R3	Burned, covered	1.46×10^4
L4	Burned, covered	3.85×10^4
R5	Burned, covered	$<2 \times 10^2$ (taken for statistical analysis as 2×10^2)

$$\text{Average}^4 = 6.13 \times 10^4$$

R2	Burned, uncovered	1.26×10^4
L3	Burned, uncovered	1.60×10^4
R4	Burned, uncovered	4.55×10^3
L5	Burned, uncovered	1.00×10^3

$$\text{Average}^4 = 8.54 \times 10^3$$

¹Number of colonies reported is the average of the results of two replicate plates counted 48 hours after plating. The cultures were taken by superficial swabbing of each site 48 hours after burning.

²Covered with "wipe on" burn dressing TS 1181-1.

³Burned with a 75° branding iron for a duration of 95 seconds.

⁴Statistical analysis by t statistic for two means, null hypothesis ($H_0: \mu_{\text{covered}} - \mu_{\text{uncovered}} = 0$) is substantiated at the 0.05 level of significance.

APPENDIX XIII

"WIPE ON" BURN COVERING STUDY: EVAPOROMETER DATA (PIG #335; NOVEMBER 11, 1981)

<u>Time</u>	<u>Site</u>	<u>Treatment</u>	<u>Cumulative Rate of Evaporation (g/cm²sec.)</u>
4 hrs. ¹	L1	Unburned, uncovered	1.27×10^{-5}
	R1	Unburned, covered	1.90×10^{-6}
	L2	Burned, covered	6.36×10^{-7}
	R2	Burned, uncovered	6.36×10^{-7}
	L3	Burned, uncovered	6.36×10^{-7}
	R3	Burned, covered	0.00
	L4	Burned, covered	0.00
	R4	Burned, uncovered	0.00
	L5	Burned, uncovered	0.00
	R5	Burned, covered	0.00
24 hrs. ¹	L1	Unburned, uncovered	2.01×10^{-6}
	R1	Unburned, covered	4.47×10^{-6}
	L2	Burned, covered	4.45×10^{-6}
	R2	Burned, uncovered	1.91×10^{-6}
	L3	Burned, uncovered	4.45×10^{-6}
	R3	Burned, covered	6.36×10^{-7}
	L4	Burned, covered	0.00
	R4	Burned, uncovered	0.00
	L5	Burned, uncovered	0.00
	R5	Burned, covered	0.00
48 hrs. ²	L1	Unburned, uncovered	8.45×10^{-6}
	R1	Unburned, covered	4.24×10^{-7}
	L2	Burned, covered	2.00×10^{-9}
	R2	Burned, uncovered	2.12×10^{-7}
	L3	Burned, uncovered	2.32×10^{-6}
	R3	Burned, covered	0.00
	L4	Burned, covered	0.00
	R4	Burned, uncovered	2.12×10^{-7}
	L5	Burned, uncovered	4.24×10^{-7}
	R5	Burned, covered	0.00

¹ Readings taken over a 60 second period at 10 second intervals

² Readings taken over a 180 second period at 10 second intervals

Please Quote: TS 1181-1
DP-1/PCL

PILOT STUDY FOR EVALUATION OF SYNTHETIC POLYMER "WIPE ON" BURN COVERING
IN MINIATURE PIGS FOR DYNATECH R/D COMPANY, CAMBRIDGE, MA
(DP-1/PCL)

Supplement I to Final Report, Dated December 14, 1981

E. M. Essigmann, Ph.D.

December 24, 1981

Ms. Connie West
99 Erie Street
Cambridge, MA 02139

Toxicol-Sisa, Inc.
763D Concord Avenue
Cambridge, MA 02138

Tel. 617/491-2496
Tlx. 951585 TOXSIS CAM

EVALUATION OF SELECTED SPECIAL STAINS

The appearance of the H & E sections of the pig skin biopsy specimens indicated that there might be more damage to the dermis than could be appreciated with standard staining techniques. It was decided to re-evaluate sections from several of the biopsies after staining with special stains designed to differentiate connective tissue fibers and muscle tissue.

Methods and Materials

Three of the pig skin biopsy samples which were described in the Final Report dated 12/14/81 (R5 from Burn Generation Study, Trial II and L5 and R5 from the "Wipe On" Burn Dressing Study) were recut and stained with three special stains: Van Gieson's Collagen Stain, Masson's Trichome Method, and Mallory's Phosphotungstic Acid Hematoxylin Method. They were evaluated at the microscopic level by Dr. James C. Murphy, D.V.M., Ph.D., of M.I.T., Department of Comparative Animal Medicine.

Results & Conclusions

The pathology report is presented in Appendix I.

The results indicate that none of the special stains were able to provide any additional information with regard to the severity of tissue damage to the dermis.

Submission of Supplement I

Submitted by:

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Ellen M. Essigmann, Ph.D.

Study Director
Department of Toxicology

Reviewed by:

Francis J. Meckler
Francis J. Meckler, Sc.D.

Department Head
Department of Toxicology

APPENDIX I

TOXICOL-SISA

Pig Burn Study

Evluation of Selected Special Stains

(Stains tested - Van Gieson's Collagen Stain; Masson's Trichome Method; Mallory's Phophotungstic Acid Hematoxylin Method)

None of the 3 stains tested provided any more definitive information related to the depth of tissue damage in the dermis of the skin, from pigs receiving skin surface burns under experimental conditions, than could not be discerned from standard hematoxylin and eosin stained sections from the same areas.

James C. Murphy, DVM, PhD
Veterinary Pathologist

JCM/bp
12/16/81

Please Quote: TS 1181-1
DP-2/PCL

MAIN STUDY FOR EVALUATION OF SYNTHETIC
POLYMER "WIPE ON" BURN COVERING IN THE MINIATURE
PIG FOR DYNATECH R/D COMPANY, CAMBRIDGE, MA

Final Report

By

Ellen M. Essigmann, Ph.D.

April, 1982

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Main Study for Evaluation of Synthetic
Polymer "Wipe On" Burn Covering in the Miniature Pig

TEST MATERIAL: Poly-E-Caprolactone (#26341) in triethylcitrate,
methylene chloride and methylacetate

TOXICOL-SISA REF. NO.: TS 1181-1

PROJECT: DP-2/PCL

SUMMARY

A main study was performed for the purpose of evaluating a "wipe on" burn covering in terms of its effect on transepidermal evaporation, microbial activity and healing in thermal burns produced in a miniature pig. The study consisted of a one pig experiment with 5 uncovered and 5 covered burn sites.

Microscopic evaluation of skin biopsies removed 48 hours after treatment did not reveal any effect of the synthetic dressing on the severity of the wound. There was significantly less microbial activity on the surface of covered burn sites relative to that of uncovered sites ($P = 0.01$). Application of the wound dressing caused a significant ($P = 0.01$) decrease in the cumulative rate of evaporation from burned sites, as determined at 48 hours post treatment.

MAIN STUDY FOR EVALUATION OF SYNTHETIC
POLYMER "WIPE ON" BURN COVERING IN THE MINIATURE PIG

TEST MATERIAL: Poly-ε-caprolactone (#26341) in triethylcitrate,
methyane chloride and methylacetate

TOXICOL-SISA REF. NO.: TS 1181-1

PROJECT: DP-2/PCL

STUDY OBJECTIVES

To evaluate, over a 48 hour period, a "wipe on" burn covering in terms of its effect on transepidermal evaporation, microbial activity and healing in thermal burns produced in a miniature pig.

Test Material

A clear, pale amber fluid in a clear glass, screw-capped jar labelled Dynatech #011280-1 was received from Dynatech R/D Co. on November 5, 1981. A small amount of the polymer appeared to have settled to the bottom of the jar. Approximately 300 ml of material was provided, along with two 1" wide natural bristle brushes to use in wiping on the dressing. The sample was designated TS 1181-1.

The test material was reported by Dynatech to consist of 40g of Poly-E-Caprolactone (#26341) dissolved in a mixture of triethylcitrate, methylacetate and methylene chloride (Appendix I).

Purity Analysis

No analyses of the sample material were performed by Toxicol-Sisa.

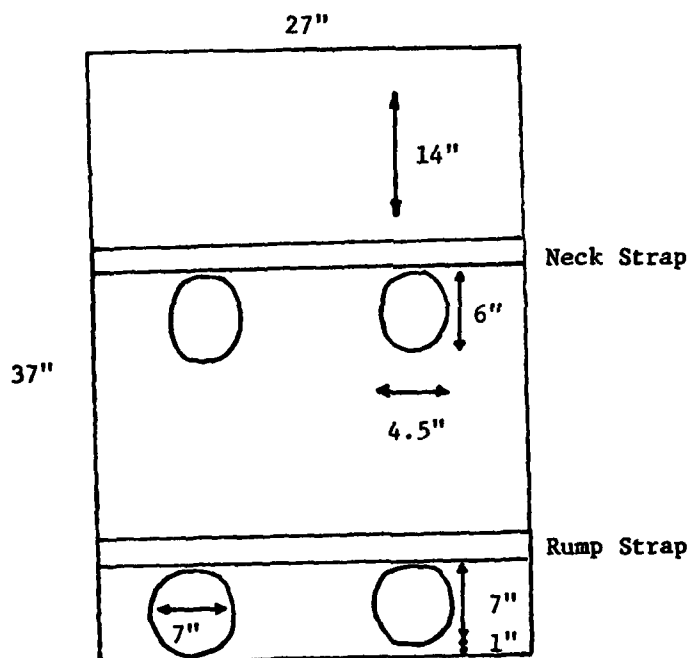
Animals

A female miniature pig of the Pitman-Moore strain, aged 14 months and weighing 29 kg, was supplied by Vita Vet Laboratories, Inc., Marion, Indiana. The acclimation period was 6 days, during which time the pig was subjected to an examination for clinical signs of disease. The animal was housed in a stainless steel dog metabolism cage under controlled temperature and humidity conditions. The pig was identified by ear tag and cage card. Approximately 500 grams of chow (Respond 2000 dog food, Agway, Inc. Syracuse, NY), mixed with water, was provided twice daily. Water (approximately two liters) was provided three times a day. The pig was weighed on arrival and on the day it was placed on study. The pig weighed 29 kg at the initiation of the study.

Materials

Pig Sling

A pig sling custom made by Bailey's Sportswear Inc., Boston, MA was constructed of heavy canvas with nylon lined leg holes and 1 1/2" wide heavy nylon straps with toothed buckles. The dimensions are given in the diagram below. The sling was attached to an angle iron frame using nylon rope threaded through a series of grommets set into the sling. The frame measured 18" wide, 59" long and 42" high.



Evaporometer

A hygrometer (HygroDynamics, Silver Spring, MD) fitted with a green humidity sensing probe (#733622) set within a plastic housing with a 25.81 cm² (2 x 2") opening and a volume of 0.213 liters was provided by Dynatech R/D, Inc.

Branding Irons

Three stainless steel branding irons with removable wooden handles were provided by Dynatech R/D, Inc. The dimensions of each iron were 2" high by 2" wide by 2" deep. Each iron weighed approximately 3 kg. Prior to use, the irons were heated to 75°C in a hot oil bath and then wiped free of oil.

Oil Baths

Glass crystallization dishes containing Molykote silicone fluid (Dow Corning Corp., Midland, MI) and electrically heated using a 10 foot coil of 1.02 OHMS/foot wire connected to an autotransformer (Powerstat, Superior Electric Co., Bristol, CT).

Anesthesia

Xylazine
Ketamine hydrochloride
Acepromazine

Study Design

This study was performed using Pig #411, which weighed 29 kg at the initiation of the study.

The day before burning, the pig's back was shaved with electric clippers, treated with depilatory cream, rinsed and dried. The skin on the pig's back was then marked with indelible ink into 12 sites approximately 2 x 2" square (Diagram I).

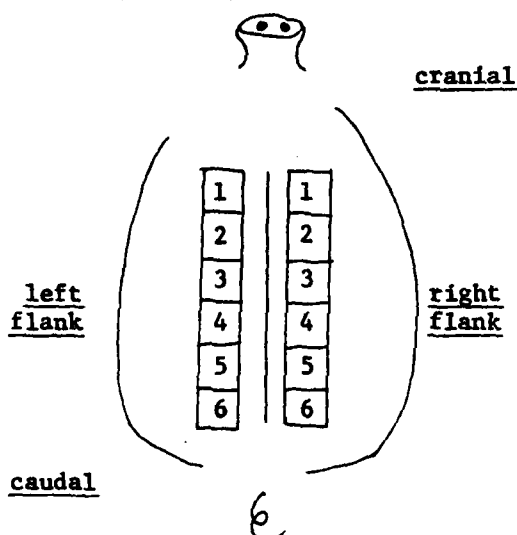
The pig was fasted overnight, weighed and then injected I.M. with a mixture of Ketamine (8 mg/lb. bodywt.) and Xylazine (4 mg/lb. bodywt.). The pig was then transferred to the sling and its rate of respiration and response to pain stimuli (jabbing of the soft tissue of the snout with a hypodermic needle) were monitored.

The marked skin sites on the pig's back were then treated as outlined in Diagram I: either left completely untreated (site R1), treated by covering with burn dressing (site L1); treated by burning with an iron heated to 75°C for 95 seconds (sites L2, R3, L4, R5, L6) or treated by burning followed by application of "wipe-on" dressing (sites R2, L3, R4, L5, R6). A detailed description of the method of burning and application of "wipe-on" dressing used in this study was reported in the final report for the pilot study DP-1/PCL ("Wipe On" Wound Dressing Study, p. 15).

The sites were described at 10 minutes after burning. A description at 48 hours after burning was not performed due to difficulty with removal of the dressing. Evaporimeter readings were taken on all sites at approximately 4, 24 and 48 hours after burning. The method used was described in the Final Report for Study DP-1/PCL ("Wipe-On" Dressing Study) except that all readings were taken over a 2 minute period and the pig was kept lightly anesthetized with a mixture of ketamine/xylazine during each of the reading periods.

Diagram #1

Burn Diagram for "Wipe On" Dressing Study Performed on Pig #411
Using TS 1181-1 (2/16/82)



	<u>Site</u>	<u>Treatment</u>
L	1	unburned, covered*
	2	burned,** uncovered
	3	burned, covered
	4	burned, uncovered
	5	burned, covered
	6	burned, uncovered
R	1	unburned, uncovered
	2	burned, covered
	3	burned, uncovered
	4	burned, covered
	5	burned, uncovered
	6	burned, covered

* covered: TS 1181-1 applied with 1" natural bristle brush

** burned: 75°C iron for 95 seconds

A portion of the "wipe on" dressing was removed from each covered site using sterile forceps. All sites were cultured using sterile cotton swabs dipped in Tryp-Soy Broth (Scott Laboratories, Fiskeville, RI). The method of culturing consisted of rolling two swabs separately over an area approximately 1 cm² in size on each site and then placing the swabs in approximately 1 ml of broth. The tubes were maintained at approximately 15°C during transportation to the microbiology lab at M.I.T. Six dilutions were prepared from each sample (10⁻¹, 10⁻², 10⁻³, 10⁻⁴, 10⁻⁵ and 10⁻⁶). Each dilution was plated onto 2 tryptic soy agar plates. Plates with between 30 and 300 colonies were counted; the average count from two plates was taken as the culture result.

Biopsy samples were taken from each site as described in the DP-1/PCL study Final Report ("Wipe On" Dressing Study) with the exception that each biopsy sample was approximately 2" long and included 1" of unburned adjacent skin and 1" of burned skin.

The pig was maintained within the sling for approximately 48 hours after burning. During this time, the pig was offered its usual diet of chow and water twice daily. An exception to this procedure was that the pig was returned to its cage for a 5 hour period on day 2 of the study (2/17/82) and allowed free access to food and water.

RESULTS AND CONCLUSIONS

General

After shaving and treating the pig's back with depilatory cream, the skin was rinsed and patted dry with paper towels. Unlike the DP-1/PCL study for the evaluation of the "Wipe On" dressing, the pig's back was not scrubbed with a nylon scrubbing pad. In the previous study, it had been observed that the dried wound cover frequently lifted away from the underlying skin which had been scrubbed to remove dead skin. It was felt that, for the present study, better adherence of the wound cover to the skin could be achieved by eliminating this scrubbing procedure. The wound cover adhered tightly to the underlying burned and unburned skin. There was no lifting up of the dried PCL film during the 48 hour observation period. This tight adherence prevented the removal of all but a small (at least 1 cm²) portion of the film from each of the covered skin sites.

Approximately 24 hours after burning, the pig refused both food and water. It was transferred from the sling back into its cage for a 5 hour period. During this time it ate and drank its usual ration.

Clinical and Histopathological Data

At 10 minutes after burning, all burn sites were reported to have a uniformly whitened appearance compared to adjacent normal skin. No evaluation was made at 48 hours due to the difficulty encountered in removing the wound cover from the surface of the covered burn sites.

The histopathology report is presented in Appendix II. A summary of the report is in Appendix III. All of the burn sites were either 2° or 2° bordering on 3° in severity, indicating full thickness necrosis of the epidermis coupled with superficial to partial necrosis of the underlying dermis. There was no apparent relationship between the presence of the wound dressing and the severity of the wound as determined by histopathological evaluation. There were equal numbers of sites rated 2° and 2°/3° within both the burned, covered and burned, uncovered treatment groups.

Microbial Assay Results

The results of the microbial assay are presented in Appendix V. The average number of colonies cultured from uncovered burn sites was 2.94×10^5 (S.E. = 2.62×10^5) versus 4.18×10^2 (S.E. = 1.2×10^2) for covered burn sites. The averages suggest an approximately 700 fold higher microbial activity present in the uncovered site compared to the covered sites. Microbial activity appears to be treatment related, with a level of significance equal to 0.01 (Mann-Whitney Rank Test). There was insufficient data (one value per treatment group) to statistically evaluate the effect of the dressing on unburned skin.

Evaporometer Data

The evaporometer data is presented in Appendices VI (individual site values according to treatment group and time) and VII (average values according to treatment group and time). At each time interval (4, 24 and 48 hours), the cumulative rate of evaporation for the uncovered sites was approximately twice that calculated for the covered sites. At the 4 hour and 24 hour time intervals, the difference between covered and uncovered was not significant ($P = 0.05$), as determined using the "paired + - statistic". There was a significant treatment related effect demonstrated at the 48 hour assessment period. The average cumulative rate of evaporation for the uncovered sites was significantly different ($P = 0.01$) from that of the covered sites (1.88×10^{-5} versus 9.78×10^{-6} g/cm² sec.).

PERSONNEL INVOLVED ON STUDY

Study Director:	Ellen M. Essigmann, Ph.D.
Director of Laboratory:	Francis J. Mecler, Sc.D.
Toxicologist:	Charles Nicholas James
Staff Veterinarian:	James Fox, D.V.M. - M.I.T. Christopher Newcomber, D.V.M. - M.I.T.
Veterinary Pathologist:	James Murphy, D.V.M. - M.I.T.
Microbiologist:	Joel Ackerman, M.S. - M.I.T.

REPORT SUBMISSION SHEET

Submitted by:

Ellen M. Essigmann
Ellen M. Essigmann, Ph.D.
Study Director
Department of Toxicology

Reviewed by:

Francis J. Mecler
Francis J. Mecler, Sc.D.
Department Head
Department of Toxicology

APPENDIX I

FORMULATION OF "WIPE ON" DRESSING DESIGNATED TS 1181-1¹

#26341 Poly-E-Caproletrone	40g
Tri Ethyl Citrate (Pfaltz & Bauer)	5.8 ml
Methylene Chloride (Howe & French)	60 ml
Methyl Acetate (Matheson Coleman & Bell)	240 ml

¹Prepared by Ms. Connie West, Dynatech R/D as Dynatech #011280-1

APPENDIX II
HISTOPATHOLOGY REPORT

Study DP2/PCL-Pigskin

Pig #411

Accession #R1

Histopathologic Findings:

This section is characterized by the presence of intact dermis and epidermis with the exception of a small area where it appears there is a sectioning artifact and the epidermis has been removed. Small accumulations of keratin, necrotic cell debris and proteinaceous material are present on the surface of this skin. All of the sections appear to be slightly overstained and there are stain precipitates especially evident in the area of the panniculus adiposus. Mild congestion, lymphocytic infiltration with separation of tissues suggestive of mild edema are present in dermal papilla.

Accession #R2 (1 & 2)

Histopathologic Findings:

This section of skin is characterized by the presence of intact dermis and epidermis at one extreme of the section with accumulations of proteinaceous material, keratin and necrotic cell debris on the surface of the intact epidermis with similar but more extensive accumulations over the areas of epidermis that have undergone necrosis. Approximately two-thirds of the section is characterized by diffuse necrosis of the epidermis with the necrosis extending through and slightly beyond the papillary portion of the dermis. A moderate degree of congestion is present in the dermis and involves vessels half-way through the thickness of the dermis. There is minimal to no evidence of leukocytic infiltration of the necrotic areas. A moderate degree of focal hemorrhage is present around hair follicles and adnexal glands in portions of the section where the necrosis is most extensive.

Accession #R3 (1 & 2)

Histopathologic Findings:

This section is characterized by intact dermis and epidermis that extends for approximately one-half of the section. There is some mild accumulation of necrotic cell debris and keratin over a portion of the intact epidermis. A moderate degree of necrosis is present throughout the remainder of the section that extends through the epidermis and partially into the dermis. The depth of extension of necrosis is similar to that observed in the previous section. A moderate degree of congestion is present in vessels throughout the zone of necrosis, but most of the congested vessels are limited to the proximal one-half of the dermis. A focal area of hemorrhage with some early necrotic changes are evident around the base of one hair follicle. A slight to moderate degree of polymorphonuclear cell infiltration is present in the necrotic portion of the dermis, especially in the dermal papilla.

Accession #R4 (1 & 2)

Histopathologic Findings:

This section is characterized by the presence of intact dermis and epidermis extending for approximately one-third of the section. Small accumulations of keratin, proteinaceous material and necrotic cell debris are focally present on the surface of the intact epidermis. Extensive necrosis is evident in the epidermis and it extends through approximately one-quarter the thickness of the dermis through the remainder of the section. Accumulations of keratin, proteinaceous material and necrotic cell debris are also focally present on the surface. A slight to moderate degree of congestion is present in vessels throughout at least half of the thickness of the dermis in most areas and occasionally involves vessels that are even deeper in other areas. Focal hemorrhage is evident around the base of a few hair follicles and adnexal glands. Changes suggestive of fibrinoid necrosis are evident in the walls of two or three vessels that are located deep in the dermis, and these vessels are surrounded by a slight to moderate

Accession #R4 (1 & 2) Continued:

polymorphonuclear cell infiltrate. Minimal evidence of leukocytic infiltration is present in the necrotic portion of the dermis and epidermis.

Accession #R5 (1 & 2)

Histopathologic Findings:

This section is characterized by the presence of intact dermis and epidermis extending through approximately one-third of the section. The remaining two-thirds of the section are characterized by the presence of necrosis which extends through the epidermis and involves the dermal papilla. In a few small areas the necrosis appears to extend even further but the majority of the section is limited to necrosis of the dermal papilla. In multiple foci there is evidence of separation of the epidermis from the dermis with accumulations of proteinaceous fluid in the clefts that are formed. A slight degree of congestion is present throughout the entire thickness of the dermis and there are focal areas of hemorrhage surrounding some hair follicles. There is evidence of fibrinoid necrosis affecting the walls of several vessels and there are accumulations of polymorphonuclear leukocytes, both within and surrounding several vessels. There is minimal to no evidence of leukocytic infiltration of the necrotic portions of the dermis.

Accession #R6 (1 & 2)

Histopathologic Findings:

This section is characterized by the presence of intact epidermis and dermis throughout approximately one-third of the section. There are sectioning artifacts in the remainder of the section which have resulted in fragmentation and sometimes removal of portions of the epidermis and dermis. Extensive necrosis which extends through the epidermis and approximately one-third of the way through the dermis is present throughout the remainder of the section. Multiple large open vesicular spaces have formed within the epidermis proper but their contents have apparently washed out during processing of the

Accession #R6 (1 & 2) Continued:

tissues. A slight to moderate degree of congestion is present throughout vessels of the dermis in the affected portions with focal areas of hemorrhage surrounding a few hair follicles and adnexal glands. Focal areas of fibrinoid necrosis of vessels and perivascular cuffing of polymorphonuclear leukocytes is also evident in the dermis. There is minimal to no evidence of leukocytic infiltration of the necrotic portions of the dermis and epidermis.

Accession #L1 (1 & 2)

Histopathologic Findings:

This section is characterized by the presence of intact epidermis and dermis throughout the entire length of the section with the exception of a small area where the epidermis is absent, but this appears to be a sectioning artifact. Small accumulations of keratin, necrotic cell debris and some proteinaceous materials are focally present on the surface of the section. The papillary portions of the dermis appear mildly edematous with a mild lymphocytic infiltration in these areas. There is no evidence of congestion of the dermal vasculature.

Accession #L2 (1 & 2)

Histopathologic Findings:

This section is characterized by the presence of intact epidermis and dermis that extends approximately one-quarter of the length of the section. The remaining portion of the section is characterized by the presence of necrosis extending through the epidermis and papillary portion of the dermis with focal extension beyond the papillary level. A slight to moderate degree of congestion is present in vessels throughout the full thickness of the dermis, and there are focal areas of hemorrhage associated with hair follicles and adnexal glands. There also appears to be early separation of the epidermis from the dermis and many of these areas contain proteinaceous fluid. In some necrotic portions of the epidermis there are large accumulations of what appear to be coccoid bacteria.

Accession #L3 (1 & 2)

Histopathologic Findings:

This section is characterized by the presence of intact epidermis and dermis through slightly less than a quarter of the section. There are small accumulations of keratin and necrotic cell debris on the surface of this portion of the section. The remaining portion of the section is characterized by the presence of necrosis which extends through the epidermis and slightly beyond the papillary portion of the dermis. Multiple areas of separation exist between the epidermis and dermis and most of these clefts are filled with proteinaceous fluid. A moderate degree of congestion is evident in vessels extending approximately half-way through the thickness of the dermis. Focal areas of hemorrhage are present associated with hair follicles and adnexal glands. There is evidence of fibrinoid necrosis of the walls of a few vessels with mild polymorphonuclear cell infiltration of the necrotic areas.

Accession #L4 (1 & 2)

Histopathologic Findings:

This section is characterized by a portion of intact epidermis and dermis at one extreme end of the section. The remaining 95% of the section is characterized by diffuse necrosis of the epidermis extending for a short distance into the underlying dermis. A moderate degree of congestion is present in dermal vessels in the proximal one-half of the dermis. Large portions of the epidermis have separated from the underlying dermis and there is extensive accumulations of proteinaceous fluid and a few polymorphonuclear leukocytes in these areas of separation. Focal areas of hemorrhage and early necrosis are evident at the base of a few hair follicles.

Accession #L5 (1 & 2)

Histopathologic Findings:

This section of skin is characterized by the presence of intact epidermis and dermis that extends approximately a quarter of the section. The remaining portion of the section is characterized by

Accession #L5 (1 & 2) Continued:

the presence of necrosis of the epidermis with focal areas of extension of necrosis into the underlying papillary layer of the dermis. Multiple areas of vesicle formation filled with proteinaceous fluid are present at the area of junction of the dermis and epidermis throughout the section. There is evidence of congestion of vessels throughout the full thickness of the dermis and there is evidence of fibrinoid necrosis in the walls of a few vessels with mild polymorphonuclear perivascular infiltration. Focal areas of hemorrhage are also present associated with hair follicles and adnexal glands.

Accession #L6 (1 & 2)

Histopathologic Findings:


This section is characterized by the presence of intact epidermis and dermis throughout approximately one quarter of the section with focal accumulations of keratin, necrotic cell debris and proteinaceous material on the surface in this area. The remainder of the section is characterized by the presence of diffuse necrosis affecting the epidermis with extension into the papillary portions of the dermis, and focally extending more deeply into the underlying dermis. Accumulations of keratin, necrotic cell debris and proteinaceous material are focally present on the surface of the necrotic portions. A moderate degree of congestion is present in dermal vessels throughout the full thickness of the dermis with mild focal hemorrhage associated with the base a few hair follicles and adnexal glands. There is minimal evidence of leukocytic infiltration of the dermis or epidermis, but there are multiple areas of separation of the epidermis from the dermis with accumulations of proteinaceous fluid in these clefts.

Summary and Conclusion

Using the following grading system:*

- 0 - No necrosis
- 1 - Epidermal necrosis
- 2 - Epidermis and papillary layer of dermis
- 3 - Epidermis with extension slightly beyond papillary level
- 4 - Epidermis and half-way through the dermis

<u>Slide</u>	<u>Grade</u>	<u>Slide</u>	<u>Grade</u>
R 1	0	L 1	0
R 2	2	L 2	2
R 3	3	L 3	2
R 4	3	L 4	3
R 5	2	L 5	2
R 6	3	L 6	2


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*Note: This grading system was modified in order to be consistent with the system used for DP-1/PCL report; a copy of the grading system used for DP-2/PCL is presented in Appendix IV.

APPENDIX III

Grading of Burns Produced in Pig #411 in "Wipe On" Dressing Study
Performed 2/16 through 2/18/82¹

<u>Site</u>	<u>Treatment</u>	<u>Burn Grade</u>
L 1	unburned, covered	0
R 1	unburned, uncovered	0
L 2	burned, uncovered	2°
R 3	burned, uncovered	2°/3°
L 4	burned, uncovered	2°/3°
R 5	burned, uncovered	2°
L 6	burned, uncovered	2°
R 2	burned, covered	2°
L 3	burned, covered	2°
R 4	burned, covered	2°/3°
L 5	burned, covered	2°
R 6	burned, covered	2°/3°

¹See Appendix IV for explanation of grading system.

APPENDIX IV

GRADING SYSTEM FOR THE HISTOPATHOLOGICAL EVALUATION OF THERMAL BURNS IN THE SKIN
OF MINIATURE PIGS¹

<u>Grade</u>	<u>Description</u>
0	Normal skin
1°	a) Partial or complete necrosis of the epidermis b) Congestion of the superficial dermal vessels
1°/2° ²	a) Same as above b) Same as above c) Separation of the epidermis from the dermis d) Exudation of leukocytes and proteinaceous fluid into area of separation
2°	a) Same as above b) Same as above c) Same as above d) Same as above e) Necrosis of the dermis at the junction of the dermis and epidermis (papillary region)
2°/3° ³	a) Same as above b) Same as above c) Same as above d) Same as above e) Same as above f) Dermal necrosis that extends beyond the papillary region but involves less than the full thickness of the dermal layer
3°	a) Same as above b) Same as above c) Same as above d) Same as above

e) Same as above

f) Full thickness dermal necrosis

¹Based on a modification of the burn classification systems discussed by Rudowski (Burn Therapy and Research, John Hopkins Union Press, Baltimore, 1976).

²1°/2° indicates a burn which is 1° bordering on 2° in severity.

³2°/3° indicates a burn which is 2° bordering on 3° in severity.

APPENDIX V

Microbiology Report on "Wipe On" Dressing Study Performed on Pig #411 (2/16 to 2/18/82)

<u>Site</u>	<u>Treatment</u>	<u>Number of Colonies</u> ¹	<u>Average</u> ⁴
L1	Unburned, Covered ²	2.30×10^2	--
R1	Unburned, Uncovered	9.20×10^3	--
L2	Burned ³ , Uncovered	1.19×10^4	2.94×10^5 (2.62×10^5) ⁵
R3	Burned, Uncovered	8.20×10^3	
L4	Burned, Uncovered	1.12×10^5	
R5	Burned, Uncovered	1.34×10^6	
L6	Burned, Uncovered	2.09×10^3	
R2	Burned, Covered	5.60×10^2	4.18×10^2 (1.2×10^2) ⁵
L3	Burned, Covered	5.0×10^1	
R4	Burned, Covered	8.2×10^2	
L5	Burned, Covered	0	
R6	Burned, Covered	3.1×10^2	

¹Number of colonies reported is the average of the results of 2 replicate plates counted 48 hours after plating.

²Covered with "wipe on" burn dressing TS 1181-1.

³Burned with a 75° branding iron for a duration of 95 seconds.

⁴ \bar{X} (\pm S.E.) = Mean \pm Standard Error

⁵Significantly different ($P = 0.01$)

APPENDIX VI

Individual Cumulative Rates of Evaporation By Treatment and Time

<u>Time</u>	<u>Site</u>	<u>Treatment</u>	<u>Cumulative Rate of Evaporation (g/cm² sec)</u>
4 hours	L1'	Unburned, Covered	1.06×10^{-5}
	R1	Unburned, Uncovered	5.72×10^{-6}
	L2'	Burned, Uncovered	1.53×10^{-5}
	R3	Burned, Uncovered	7.00×10^{-6}
	L4	Burned, Uncovered	6.36×10^{-6}
	R5	Burned, Uncovered	1.27×10^{-6}
	L6	Burned, Uncovered	6.36×10^{-7}
	R2	Burned, Covered	1.91×10^{-6}
	L3	Burned, Covered	8.91×10^{-6}
	R4	Burned, Covered	1.27×10^{-6}
	L5	Burned, Covered	6.36×10^{-7}
	R6	Burned, Covered	0
	L1	Unburned, Covered	0
	R1	Unburned, Uncovered	1.91×10^{-6}
	L2	Burned, Uncovered	2.04×10^{-5}
	R3	Burned, Uncovered	1.72×10^{-5}
24 hours	L4	Burned, Uncovered	1.46×10^{-5}
	R5	Burned, Uncovered	3.82×10^{-6}
	L6	Burned, Uncovered	1.91×10^{-6}

Mechanical abrasion present on lesion surface; lesion produced when pig was placed in sling prior to burning.

APPENDIX VI

Individual Cumulative Rates of Evaporation by Treatment and Time

<u>Time</u>	<u>Site</u>	<u>Treatment</u>	<u>Cumulative Rate of Evaporation (g/cm² sec)</u>
24 hours	R2	Burned, Covered	8.27×10^{-6}
(cont.)	L3	Burned, Covered	1.27×10^{-5}
	R4	Burned, Covered	7.00×10^{-6}
	L5	Burned, Covered	2.54×10^{-6}
	R6	Burned, Covered	0
48 hours	L1	Unburned, Covered	0
	R1	Unburned, Uncovered	3.82×10^{-6}
	L2	Burned, Uncovered	2.42×10^{-5}
	R3	Burned, Uncovered	2.48×10^{-5}
	L4	Burned, Uncovered	2.29×10^{-5}
	R5	Burned, Uncovered	1.21×10^{-5}
	L6	Burned, Uncovered	1.02×10^{-5}
	R2	Burned, Covered	1.14×10^{-5}
	L3	Burned, Covered	1.65×10^{-5}
	R4	Burned, Covered	1.08×10^{-5}
	L5	Burned, Covered	8.27×10^{-6}
	R6	Burned, Covered	1.91×10^{-6}

APPENDIX VII

Average Cumulative Rate of Evaporation By Treatment and Time

<u>Time</u>	<u>Treatment</u>	<u>Average Cumulative Rate of Evaporation (g/cm² sec)</u>
4 hours	Burned, Uncovered	6.11×10^{-6} (2.63×10^{-6}) ¹
	Burned, Covered	2.54×10^{-6} (1.62×10^{-6})
24 hours	Burned, Uncovered	1.16×10^{-5} (3.38×10^{-6})
	Burned, Covered	6.10×10^{-6} (2.22×10^{-6})
48 hours	Burned, Uncovered	1.88×10^{-5} (3.16×10^{-6}) ²
	Burned, Covered	9.78×10^{-6} (2.37×10^{-6}) ²

¹Average (standard error), n = 5 for each group

²Significantly different at the 0.01 level of significance

Section 11

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) The current state of burn-wound therapy and treatment was investi- gated. Literature sources of the period 1977 - August, 1981, were surveyed, yielding 283 references discussed in the review of burn wound treatment and healing. Interviews were held with eight researchers currently active in the development of materials for burn wound coverings. Their activities and opinions were summarized. (cont. on reverse side)		

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20. Abstract (continued)

The adherence of four wound coverings to areas of skin excision on rats was measured. Quantitative measures of adherence were obtained and differences in fluid accumulation under the coverings were observed.

A poly- ϵ -caprolactone covering developed for the Navy was tested in two pig experiments and evaluated by means of bacteriology, histopathology, and pervaporation rates.

A pilot study was performed for the purpose of developing and evaluating methods for performing an efficacy study on a "wipe on" burn dressing. The methods developed included a procedure for anesthetizing the pig during the burning procedure, a method for restraining the pig during the observation period of 48 hours, a method for generating burns which were reproducibly between second and third degree in severity, methods for grading the burn wounds at the clinical and histological level, methods for evaluating the microbial activity on the surface of the burn wound, a method for applying the "wipe on" dressing, and a method for measuring the rate of evaporation from the skin surface. The methods were described and evaluated and suggestions were made for improving the methodology in future burn studies. Preliminary data, based on a one pig study with four covered and four uncovered burns, did not support a significant difference between covered and uncovered wounds evaluated 48 hours after burning.

The second pig experiment compared 5 covered and 5 uncovered burn sites. There was significantly less microbial activity on the surface of covered burn sites relative to that of uncovered sites ($P = 0.01$). Application of the wound dressing caused a significant ($P = 0.01$) decrease in the cumulative rate of evaporation from burned sites, as determined at 48 hours post treatment. Microscopic evaluation of skin biopsies removed 48 hours after treatment did not reveal any effect of the synthetic dressing on the severity of the wound.

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